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PATHOLOGIC ASPECTS OF POSTERIOR PROTRUSIONS OF THE INTERVERTEBRAL DISKS

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One hundred protruded intervertebral disks were studied. These had been removed by neurosurgeons of the Mayo Clinic from 94 patients operated on in the period from 1922 to 1937 because of compression of the cord and nerve root. True primary tumors of disks and tumors involving disks secondarily were not included in this report.[1] Each specimen was examined macroscopically immediately after its removal. The microscopic examination was done after fixation of the specimen in solution of formaldehyde U. S. P. (diluted 1 to 10) for at least twenty-four hours. The specimens, embedded in paraffin, were cut in the usual manner, and the sections were stained with hematoxylin and eosin as a routine. Because of their unevenness and hardness, the specimens were difficult to cut. In many cases several sections were made, representing various parts of the protrusion. In selected cases special stains were used: Mayer's mucicarmine stain, to reveal chondromucoid substance; Best's carmine stain, to show glycogen in notochordal cells, and Van Gieson's stain, to show fibrils of connective tissue.

The age distribution of the 94 patients (table) was not unusual for patients who have protrusion of an intervertebral disk. The average age was 38 years. Eighty per cent of the patients were men. The site of the protrusion likewise was not unusual. It is likely that the findings in this group of 100 persons with protrusion of an intervertebral disk will not differ from those in the larger group in which operation has been performed at the Mayo Clinic up to the present.[2]

From the Section on Neurosurgery, the Mayo Clinic.

1. Adson, A. W.; Kernohan, J. W., and Woltman, H. W.: Arch. Neurol. & Psychiat. **33**:247, 1935.

2. Love, J. G., and Walsh, M. N.: J. A. M. A. **111**:396, 1938.

MACROSCOPIC OBSERVATIONS

Grossly we can distinguish two different types of posterior protrusion of the intervertebral disk removed at operation. The tissue may be either in a single dense piece or in several fragments. These two forms of removed tissue correspond to the different types of protrusions[3] which are found at operation. The characteristic appearance of the tissue removed in one piece is that of "wet rolled up blotting paper" or of "chewed up chalk." The size of the part removed in this series of cases ranged from that of a bean to that of a hickory nut (from 1 to 2.5 cm. in diameter). The size of the removed tissue did not correspond to that of the tumor in situ, because pieces which lay inside the normal area of the disk were removed with the protruded portion. The shape of the protrusion in situ is hemispheric or oval. The tissue loses this shape on removal; the fibrous elements which are compressed broaden out; in some of our cases it was possible to stretch the removed part to 8 cm. The tissue in general is firm, but there are usually small soft parts in it. The appearance of the various portions varies with the consistence of the tissue. The denser portions are made up of fibrous elements and frequently reveal a fine lamellar structure. One part of the border may be smooth for a short extent and the other marginal parts fringed. There are usually one or more pointed processes, which are smooth and shiny and do not look fibrous. These are the softest parts of the protruded tissue. In certain of our cases these processes had the appearance of little synovial fringes (fig. 1 *A*). At operation these processes were found to extend deep into the disk.

The other type of protrusion cannot be removed so easily, and it is necessary to cut or to use the rongeur to remove all of it. There may be adhesions to the disk or to surrounding structures, such as the vertebral body, the dura mater or the ligament. Such protrusions can seldom be removed in one piece. The removed mass consists of several fragments of different sizes and shapes. The number of fragments in the various cases in this series in which the tissue was in fragments varied from 2 to 11. The size of the various pieces varied accordingly. In some cases all of the pieces were about the same size; in others the largest piece was almost as large as the entire protrusion, and various small pieces made up the rest of the mass. The shape of the individual fragment therefore varies markedly and is meaningless, as it may be due to an artefact. Besides the variation of consistence mentioned previously, bony-hard parts are present in some fragments. These areas are small pieces of vertebra which have been removed with the protruded portion of the disk. The various fragments show the same differences as the tissue removed in one piece.

3. Love, J. G., and Camp, J. D.: J. Bone & Joint Surg. **35**:776, 1937.

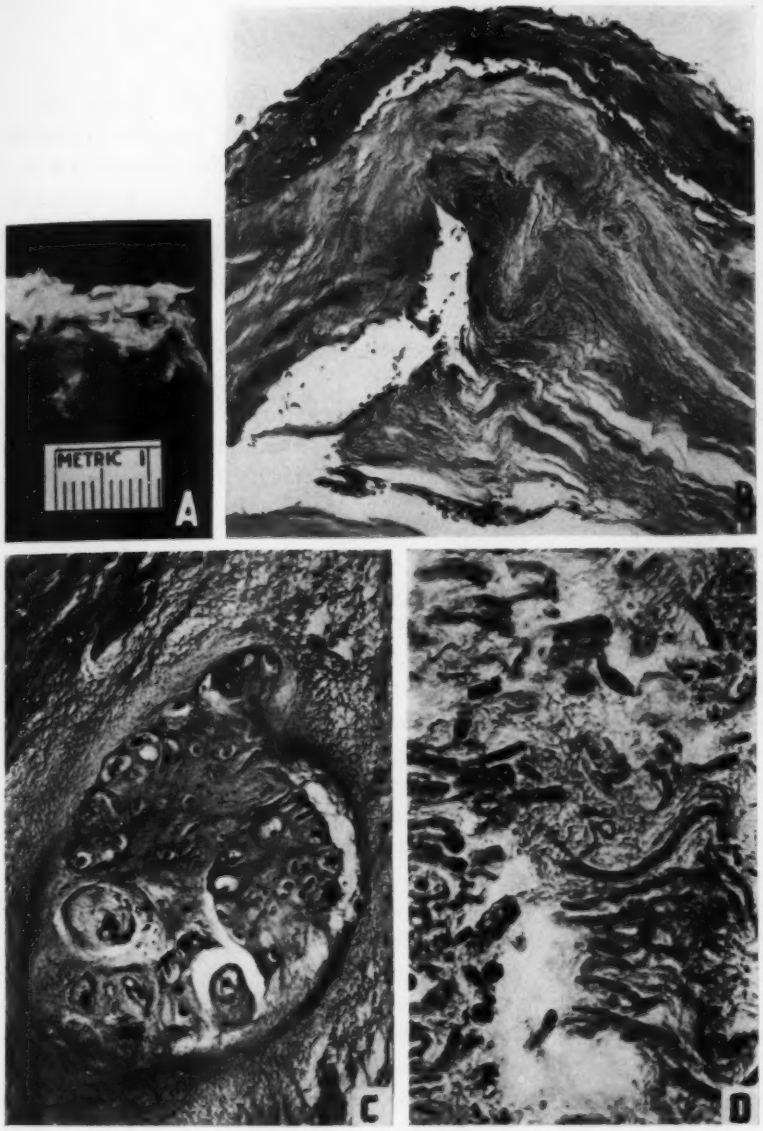

Fig. 1.—*A*, protruded portion of the fourth lumbar intervertebral disk, removed in one piece; the soft pointed processes with smooth, shiny surfaces extended into the central parts of the disk. *B*, bulging of the entire mass consisting of the outermost portion of the annulus, which is composed of parallel connective tissue fibers, and the underlying parts of the annulus; × 90. The section is from the protruded portion of a lumbosacral disk of a man 33 years of age. The structural changes in the annulus consist of an irregular course of fibrils, fissures and unusually rounded cells. *C*, large island of cartilage cells in an advanced state of degeneration; the capsule of the cartilage is indistinct and partly broken; the cell outlines are vanishing; karyorrhexis is seen; × 240. The section is from a protruded portion of the second lumbar disk of a woman 59 years of age. *D*, degenerated fibrils of an annulus; × 400. The section shows short, swollen, broken-up and irregular fibrils and hyaline masses from a protruded portion of the fourth lumbar disk of a man 23 years of age.

It is evident from this description of protruded tissue that it is made of various portions of the disk and is not unaltered in all cases. Various authors [4] have emphasized the fibrous character of the protrusion, but from this gross description no conclusion can be drawn about the changes which may have taken place in the tissue. As the outer portion of a normal disk is made of dense connective tissue, it is easy to understand the fibrous character of some of the fragments or pieces of a whole protrusion without assuming any radical pathologic change in the nucleus pulposus or fibrocartilaginous annulus fibrosus. In our material we found that frequently the marked fibrous character of separate pieces of a protrusion was caused by a relatively large admixture of the normal external portion of a disk. In the same way the separation of a protrusion into bits really has no microscopic significance.

MICROSCOPIC OBSERVATIONS

Our first interest lay in the composition of the protrusion that extended from the various portions of the normal disk. The intravertebral prolapses of the disk and the posterior protrusions which had not given rise to symptoms and which came to attention only at necropsy were usually made up chiefly, if not exclusively, of the nuclear portions of the disk.[5] In our surgical material there was not a single specimen in which annular parts of the disk were not present also. The relationship of these two elements was variable; sometimes the annular and sometimes the nuclear portions predominated. This fact is of little consequence, as the line of demarcation between these two structures is indefinite. In many cases portions of the outermost annulus with its typical connective tissue fibrils and vessels were found. This finding disclosed that we were really dealing with a rupture of the annulus from bulging of the parts behind it. Only in a few cases were the outer portions of the annulus in close relationship to the lamellar elements just beneath it (fig. 1 *B*); more often this portion represented a separate fragment. Remnants of notochordal tissue were found in numerous cases. The notochordal cells were arranged in large or small irregular areas as a rule at the edge of the specimen or in fissures in the protrusion. Most of the notochordal cells stained positively for glycogen. Stains for mucus revealed this substance in some notochordal cells and showed it in great quantities around the cartilage cells of the nuclear portion.

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4. Alajouanine, T., and Petit-Dutaillis, D.: Presse méd. **38**:1657 and 1749, 1930. Love, J. G.: Proc. Staff Meet., Mayo Clin. **11**:529, 1936. Mixter, W. J.: Ann. Surg. **106**:777, 1937.

5. (*a*) Andrae, R.: Beitr. z. path. Anat. u. z. allg. Path. **82**:464, 1929. (*b*) Beadle, O. A.: The Intervertebral Discs: Observations on Their Normal and Morbid Anatomy in Relation to Certain Spinal Deformities, Medical Research Council, Special Report Series, no. 161, London, His Majesty's Stationery Office, 1931.

Structural Changes.—In almost all cases the structure of the annulus fibrosus had undergone more or less definite change. The lamellae retained only for a short distance their normal regular course; then they broke off or followed a tortuous course. The cells of the annulus were often unusually round, and often abnormally large groups of cells were found. The changes in the course of the lamellae were probably a purely mechanical result of the bulging and rupture of the annulus. The change in the shape of the cells probably was due to changes in the functional stress on them.[6] The forces active on the lamellar elements of the protruded disk must have been changed markedly, and these then produced the changes in the cells described through their long-continued effect. In the nuclear parts structural changes were less definite; the course of the fibrils remained normal, the cells, which indeed have no characteristic form, did not seem to be definitely changed except for the relatively frequent occurrence of large cartilage islands.[7] In those areas the difference in the demands made on them was more quantitative than qualitative. Large and small irregular fissures were an almost constant finding in both the nuclear and the annular parts (fig. 1 *B*).

Degenerative Changes.—The normal disks of older men ordinarily show signs of marked degeneration.[8] There are definite individual differences both in the time of appearance and in the degree of the changes. For this reason it is difficult to evaluate correctly the degenerative changes in a protrusion if, as in our purely operative material, it is impossible to draw comparisons with the rest of the involved disk or with other intervertebral disks of the same patient. In spite of this uncertainty the occurrence of degenerative changes was worthy of note. Degeneration of a slight or moderate degree could be found in practically all protrusions.[9] Thirty-two of our 100 specimens showed advanced degeneration. These specimens were from patients in all the different age groups, and the percentage distribution was characteristic (table). Although degenerative changes appeared most frequently in the specimens from patients who were in the age group of from 51 to 60 years, marked degeneration of the protruded portion was not infrequent in specimens from young persons.

The degenerative changes involved the cartilage cells, the fibrils or both. Degeneration of the cartilage cells was much more frequent,

6. von Möllendorff, W: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 2, pt. 2, pp. 40 and 225.

7. Elsberg, C. A.: Bull. Neurol. Inst. New York **1**:350, 1931.

8. Schmorl, G., and Junghanns, H.: Fortschr. a. d. Geb. d. Röntgenstrahlen **43**:1, 1932.

9. Barr, J. S.; Hampton, A. O., and Mixter, W. J.: J. A. M. A. **109**:1265, 1937. Love and Camp.[8]

being present in 27 cases. There occurred not only evanescent degenerative changes of single cells [6] but also marked degeneration in islands of cartilage. The outlines of the separate cells in these islands of cartilage disappeared; the nuclei revealed karyorrhexis or pyknosis, and sometimes they had disappeared. All portions of the capsule of the cartilage were not intact; basophilic "halos" were sometimes extensive (fig. 1 *C*).

Degeneration of Fibrils.—This occurred usually with degenerative changes of the cells in the outermost portions of the annulus. The single fibrils were short, thick and swollen; some were broken off, some took an irregular course and some were coiled. Occasionally hyaline patches of varying size were found in which the fibriilar structure was no longer recognizable (fig. 1 *D*).

Fibrosis.—Fibrosis of the nuclear portion occurs frequently in normally placed disks. It represents the end stage of various pathologic changes and does not appear until blood vessels have invaded the field.[8]

Percentage Distribution of Patients at Time of Removal of Protruded Intervertebral Disks, Related to Percentage Distribution of Pathologic Changes and Presence of Notochordal Remnants in the Disk, in Age Groups

Age, Yr.	Cases	Pathologic Changes			Notochordal Remnants
		Degeneration	Edema	Fibrosis	
20-30	26	20	43	21	14
31-40	35	24	24	38	28
41-50	26	26	28	24	24
51-60	13	30	5	17	33
Specimens	100	32	22	28	28

Workers have remarked frequently that regressive fibrotic changes may take place in a protrusion of an intervertebral disk.[4] In our material there was fibrosis of different grades in 28 specimens. In every one of these the nuclear portion of the protrusion showed definite changes, but only rarely was one encountered in which the annular portion showed fibrotic changes. In no instance was fibrous tissue completely substituted for the fibrocartilaginous structures of the intervertebral disk.

In 14 specimens the fibrosis was related to remnants of the notochord. At the edge of the specimen or along fissures in the tissue there were areas of varying extent of notochordal tissue. The cells of the notochord occasionally were degenerated. Near these cells and mixed up closely with them were connective tissue cells with delicate narrow dark nuclei, without any vacuoles. On the margins toward the fibrocartilaginous tissue the fibrous cells showed a marked tendency toward invasion. The margin between the two types of tissue was irregular and indefinite, and oftentimes connective tissue cells were scattered deeply in the cartilage. In cases of advanced fibrosis young capillary vessels were not rare. In cases in which fibrosis was most marked the edges and the fissures

in the tissue of the protrusion were lined by dense fibrous tissue, and in a few places small rests of notochordal cells were found. Near the fibrocartilaginous elements the fibrous tissue became thinner and thinner, and finally there were only scattered fibrocytes in an otherwise normal or degenerating fibrocartilage. The close relationship of the fibrosis to the rests of the notochord was most remarkable. Notochordal cells, according to Linck,[10] pass through various stages during their life, and in the third stage they are very similar to connective tissue cells. In many of the previously described cases in our material we used the Van Gieson stain, which demonstrated plainly the presence of connective tissue fibrils in the fibrotic areas. We can say, therefore, that rests of the notochord in the protruded portion of the disk are often the point of entry for fibrous changes. Whether the fibrocytes come from the outside or are derived from the notochordal cells cannot be decided (fig. 2*A*). This finding was made by Alpers, Grant and Yaskin,[11] also, but these authors regarded it as a transition of immature, undifferentiated cells into cells resembling cartilage cells in a tumor.

In 14 cases we found fibrosis which apparently had no relationship to notochordal rests. Foci of fibrocytes of varying size were present in otherwise normal or degenerating fibrocartilaginous tissue. At their edges these fibrous nests showed a tendency to invade the surrounding tissue. Their border was indistinct and irregular. The arrangement of the fibrils likewise was irregular. In the center of the nest the tissue was much denser, and it became looser toward the exterior, thereby giving the appearance of typical foci of fibrosis. The Van Gieson stain demonstrated connective tissue fibrils in great numbers. In cases in which these changes were advanced, young capillaries were present.

The portion of the protrusion which did not contain fibrotic changes was in some instances normal and in others markedly degenerated. Both types of fibrosis showed nothing characteristic if considered from the point of view of the various age groups (table). Whereas many fibrotic protrusions had to be removed at operation with a rongeur, others could be taken out in one piece without difficulty. In fact, some of them popped out.[8]

Edema.—In 22 cases there was definite edematous swelling of the protrusion. On viewing the sections of such protrusions the edematous character was plain to the naked eye because of pale staining with hematoxylin and eosin. On microscopic examination we found the stained elements widely separated. In the annulus the lamellar structure was plainly recognizable by this separation. In the nucleus pulposus the

10. Linck, A., quoted by Schwyzer, A.: Minnesota Med. **20**:15, 1937.
11. Alpers, B. J.; Grant, F. C., and Yaskin, J. C.: Ann. Surg. **97**:10, 1933.

interspaces of the network structure were wider. Cells were comparatively less numerous. In the annular portion they were markedly rounded. The nuclei seemed relatively small, and the protoplasm was completely clear and transparent except for a small rest. In the nuclear

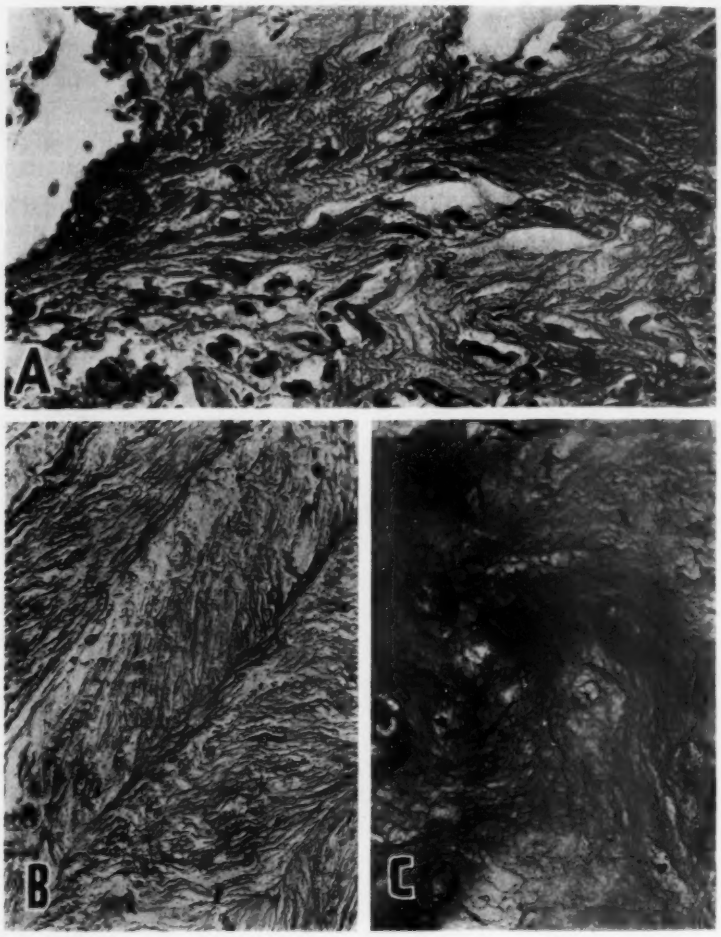

Fig. 2.—*A*, fibrosis and notochordal remnants of the protruded portion of the fourth lumbar disk of a woman aged 51 years; × 340. Besides the typical physaliferous cells, the tissue shows connective tissue cells, a dense area at the edge of the fissure and an invasive tendency of the fibrous tissue. *B*, marked edema of the annulus fibrosus in a protruded portion of the fourth lumbar disk of a woman 26 years of age who had had recurrent symptoms for four years and whose last attack had begun six months prior to laminectomy; the fibrils are normal; the rounded cells contain clear protoplasm; × 40. *C*, nuclear portion of a protrusion of the fourth lumbar disk of a man 32 years old who had had symptoms for three years and an exacerbation one month prior to laminectomy. Note the degenerating cartilage cells; × 200.

part the edematous change in the cells was less marked. The fibrils of the annulus and nucleus took no part in the edematous swelling. They were unchanged, delicate and fine, but widely separated. Various sections through the tissue from one protrusion showed edema throughout, although it was not equally well marked in all portions. Edema of this type occurred in 11 cases, with marked degeneration of the cells (fig. 2 *B* and *C*). Little or no attention has been paid previously to this point. In our material marked edema was a frequent occurrence, and slight edema was present in the majority of cases. The age of the patients from whom the specimens exhibiting marked edema had been removed was significant (table). In 20 of our 22 cases attacks of pain lasting for from three weeks to six months had occurred immediately before registration at the clinic. Attacks of varying degree prior to these had ceased. In the other 2 cases the history was somewhat indefinite, but there had been symptoms for three and two years, respectively. These facts lead to the supposition that edema may be the cause of the recurrent attacks or exacerbations of pain. In many of the cases in which repeated painful attacks occurred over a period of years, a relatively small protrusion was found at operation, so that it seems likely that in some cases at least the repeated attacks were the result of recurring edema of the protruded portion of the disk. Edematous swelling and its subsidence under altered conditions, such as rest in bed, seem to offer one explanation of the intermittency of pain in these cases.

The marked capacity of the intervertebral disk, particularly of the nucleus, of the young person to swell is well known and definite.[8] It has been demonstrated that the disk can swell to twenty times its normal size when placed in water.[12] The nucleus is kept to its normal size by the various forces acting on it from all sides. If these forces are decreased, the nucleus swells by absorption of fluid.[13] In a case of protrusion the resistance to expansion of the protruded portion is naturally decreased, and swelling of the nuclear element, provided its capacity to swell is unaltered, is to be expected. In our material we saw swelling not only in the nuclear parts but also in parts of the protruded annulus. It is therefore very likely that displacement of a portion of the intervertebral disk results in circulatory changes favoring the development of edema which will involve the nucleus and the annulus. After a time this edema may recede and may recur later if opportunity is offered. Accidental changes in the position of the protrusion relative to its surroundings as a result of activity of the patient may represent such an opportunity. The edema seems therefore to be

12. von Puky, P.: Arch. f. klin. Chir. **188**:171, 1937.

13. Beadle.[5b] Schmorl and Junghanns.[8]

the effect of the natural tendency of the nucleus to absorb fluid and of the circulatory changes resulting from the displacement of the tissue.

Such a process reminds us of the result of Ribbert's [14] early experiments. After drilling or cutting a hole in the anterior part of the disk he saw small "tumors" develop, which increased in size for about fifty-two days and then stayed about the same size or, as Leopold and Zahn believed, decreased again. Ribbert called attention to the occurrence of large vacuoles in the cells, which were largest when the swelling had reached its maximum. A gradually increasing and then receding edematous swelling of the protruded parts could easily explain these findings and would be in accord with the observations made on our material.

We have mentioned that edema of the protrusion may be of clinical significance. This edema also may account for the well known discrepancy between the age distribution of the patients who have had clinical symptoms from protruded intervertebral disks and who have undergone operation and that of persons whose protrusions have been observed incidentally at necropsy. In old persons the capacity of the nucleus pulposus to swell is markedly decreased owing to the frequent degenerated, dried, fibrotic or crushed character of this part.[8] Protrusions of the intervertebral disks in such persons have, therefore, much less tendency to become edematous, and for this reason they probably produce no symptoms.

SUMMARY

Posterior protrusions of the intervertebral disk causing symptoms which lead to operation are composed of all parts normally found in the unprotruded intervertebral disks, the annulus lamellosus, including its outer parts, and the nucleus pulposus with its occasional remnants of the notochord. The tissue of the intervertebral disk is almost invariably altered in the protrusion. The most common and constant changes in it consist of alterations of the normal architecture.

Degenerative changes, too, are seen commonly. Advanced degeneration is more frequent among patients in the older age groups. Marked degeneration of the cartilage cells is much more common than degeneration of the fibrils.

Fibrosis may occur in the form of proliferating fibrous tissue or it may be in close relationship to remnants of the notochord. In both instances the fibrous tissue tends to replace the normal fibrocartilaginous structures of the protruded portion of the disk.

Edema of the protruded part of the intervertebral disk is a most important and frequent finding. It may involve the annulus as well as

14. Ribbert: Verhandl. d. Cong. f. inn. Med. **13**:455, 1895.

the nucleus and is more frequent in young persons. The edema of the protrusion must be considered as a result of the capacity of the nucleus to swell if the normal forces keeping it in place and shape are decreased. At the same time it may be helped or held back by circulatory changes resulting from the displacement of the protruded part. The occurrence of edema may result in exacerbation of the clinical symptoms. Such an exacerbation, however, may subside spontaneously or with conservative treatment.

From this study it is not possible to draw any conclusions about an etiologic relationship of notochordal rests to protrusions of intervertebral disks.

PHAGOCYTOSIS OF TRYPAN BLUE IN RATS OF DIFFERENT AGE GROUPS

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It is generally recognized that man and animals manifest, as they grow older, increasing natural resistance against many kinds of infectious agents.[1] It appears likely, in spite of strong evidence to the contrary in a number of diseases, that this gradually developed natural resistance is often largely a matter of constitutional maturation and that exposure to a specific disease agent is not essential in order that man or animals may acquire resistance with age. As yet, the precise cause of the greater resistance of older animals is obscure, although much experimentation has been done on the question. It has long been known, for example, that older animals respond more powerfully in the production of serum antibodies than do younger ones after parenteral administration of antigenic substances. A difference in response analogous to that to an artificial stimulation might be expected after exposure to a natural infecting agent. Jungeblut and Engle [2] found experimentally that hormonal factors are of significance in the natural acquisition of resistance. Indeed, in discussing the naturally acquired resistance of adult persons against poliomyelitis, they [2a] stated ". . . epidemiologic observations and experimental facts can be reconciled with a point of view emphasizing the normal endocrine balance characteristic of mature age as a major source of this protection." [3]

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1. The literature has been reviewed comprehensively by G. P. Ssacharoff (Ergebn. d. allg. Path. u. path. Anat. [pt. 2] **22**:201, 1927-1928) and by L. Baumgartner (Yale J. Biol. & Med. **6**:403, 1934).

2. Jungeblut, C. W., and Engle, E. T.: (*a*) J. A. M. A. **99**:2091, 1932; (*b*) J. Exper. Med. **59**:43, 1934.

3. Other mechanisms to explain observed differences in the resistance of animals of different age groups to infectious agents have been suggested. A. O. Foster (Am. J. Hyg. **24**:109, 1936) expressed the belief that age resistance is linked with the natural age curve of the hemoglobin level of a given host, especially since he was able to note an inverse correlation between the resistance of dogs and cats to hookworm infection and the anemia occasioned by hemorrhage or by feeding a milk diet, which is deficient in iron. A. O. Foster and W. W. Cort (ibid. **21**:302, 1935) found that young dogs are more susceptible than older dogs to the effects of a deficient diet in decreasing the natural resistance to Ancylostoma canium.

It seems likely that the endocrine balance of an animal, as well as its improved capacity with age to produce specific antagonistic substances, is of fundamental importance in the natural acquisition of resistance. But it seems probable as well that the enhanced resistance of the older animals is in part dependent on a more effective functioning of the phagocytic cells of the older body, especially since, as Gay [3a] pointed out, "body cells, rather than body fluids, are the first and the last factors in the defense processes against micro-organisms."

There is a considerable literature indicating that significant changes do occur in the reactive capacity of the defense cells as animals mature. For example, negative or only slight reactions are noted in the skin of infants given injections of such nonantigenic substances as turpentine, iodoform, mustard oil and other cutaneous irritants, whereas the skin of older persons reacts strongly to these substances (Adelsberger;[4] Tachau [4a]). Similarly, the skin of young infants does not react to large injections of diphtheria or scarlet fever toxin, even in the absence of demonstrable antitoxin (Schick test and Dick test), although the skin of somewhat older children does respond (von Gröer and Kassowitz [5]). Furthermore, Sarles [6] has made the significant observation that old dogs experience a severe skin reaction, with prolonged inflammation, to the penetration of the dog hookworm, Ancylostoma canium, in contrast to the slight, transient effects in young dogs. The larvae of the hookworm are retained in the skin longer in old dogs than in young ones, and sections reveal partial disintegration of these parasites in the older animals.

An observation by Duca [7] in this laboratory has thrown further light on the increasing reactivity of the protective cells as animals become older. Duca noted that nursling rats, which are frequently killed by Trypanosomia lewisi, show no change either in the total number of leukocytes or in the monocyte percentage after infection, whereas older rats, beginning very soon after being weaned, manifest a sharp rise in both the total leukocyte count and the monocyte percentage after infection, these responses being apparently related to the resistance which the older animals exhibit against T. lewisi. This observation has seemed to me to be of considerable significance and has led me to investigate further the general problem of naturally acquired resistance, especially with the purpose to determine the role played by the phagocytic cells in this resistance.

3a. Gay, F. P.: Arch. Path. **1**:590, 1926.

4. Adelsberger, L.: Ztschr. f. Kinderh. **43**:373, 1927.

4a. Tachau, P.: Ztschr. f. Kinderh. **38**:638, 1924.

5. von Gröer, F., and Kassowitz, K.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **30**:154, 1920.

6. Sarles, M. P.: Am. J. Hyg. **10**:683, 1929.

7. Duca, C. J.: Am. Heart J., to be published.

The present paper offers the results of an attempt to determine the relative capacity of the cells of rats of different age groups to phagocytose inert particles injected into these animals. It was hoped to find through the use of trypan blue whether the phagocytic function of these cells becomes more effective as the animals approach maturity. If such an improvment in function was found it was considered these same cells would be shown, by analogy, responsible in part for the enhanced resistance which older animals manifest against living infectious agents.

METHODS

Suspension of Trypan Blue.—Trypan blue was prepared for injection by suspending the dry powder in distilled water, in a concentration of 1 per cent. The suspension was sterilized in the autoclave prior to being injected.

Strain of Rat Used.—All rats were of the Sherman strain and were propagated in the Department of Animal Care of the College of Physicians and Surgeons. They were maintained on a diet consisting of the following foods in the percentages given: hulled oats (15), whole wheat (15), yellow corn (15), shelled barley (15), soybean meal (15), powdered whole milk (10), commercial dried meat scraps (Swift's) (10), green alfalfa meal (2), sodium chloride (2) and calcium carbonate (0.5).

Experimental Procedure.—Rats of successive age groups to maturity were used in this experiment: 7 were 6 days old, 5 were 10, 3 were 15, 4 were 18, 3 were 23, 3 were 40, and 2 were 60 days old. Half of these animals received by intraperitoneal injection 0.1 cc. of the suspension of trypan blue per 10 Gm. of body weight. The remainder were given twice this dose. Eighteen hours later, all of the rats were killed with ether, and pieces of liver, spleen, lung, brain, cartilage (xiphoid process) and skin removed and placed in Bouin's[8] fixative. The tissues were embedded in paraffin, sectioned and stained lightly with eosin only.

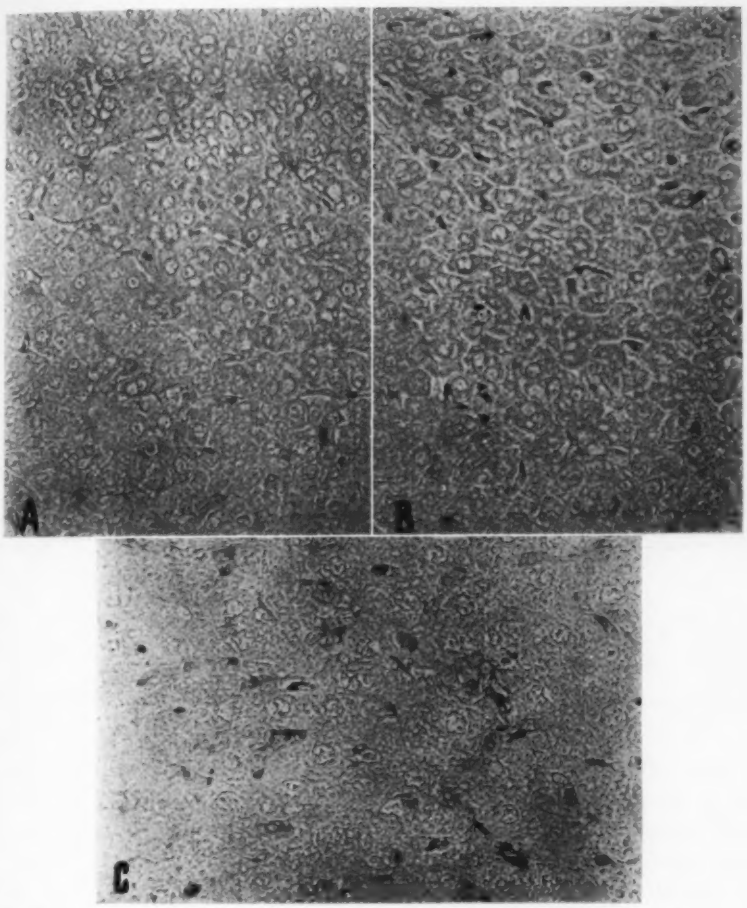
OBSERVATIONS

The examination of the prepared sections revealed no significant difference in the phagocytic capacity of the cells of any of the organs of the various age groups except the liver. In the liver, however, well marked and consistent differences were observed. Not only did more Kupffer cells in the livers of the older rats contain particles of the dye, but larger numbers of particles were to be found within each phagocytosing Kupffer cell of the older animals. The observed difference is fairly well brought out in the figure, in *A*, *B* and *C*.

An attempt was made to estimate quantitatively the difference seen in the rats of different age groups. The total number of Kupffer cells which contained any trypan blue particles whatsoever was determined in 25 representative oil immersion fields of each slide, the oil immersion objective being used in order to recognize unmistakably the individual

8. The formula is: Saturated aqueous solution of trinitrophenol, 75 parts; solution of formaldehyde U. S. P., 25 parts; glacial acetic acid, 5 parts.

particles of the dye. The quantitative results substantiated the impression given by a more superficial perusal of the slides, smaller numbers of cells, with fewer particles per cell, being observed in the animals less than 18 days old than in those over this age. It appeared, furthermore, that by the time an animal had reached 18 days of age its Kupffer



Sections of the livers of rats of different age which had received injections of trypan blue. They show the relative amount of phagocytosis by Kupffer cells. Stain, eosine only; magnification, about × 500. *A* represents rat 10, 10 days old; *B*, rat 17, 18 days old, and *C*, rat 26, 60 days old.

cells had attained their full capacity for phagocytosis, since with further development rats showed little or no continued improvement in phagocytic potentialities. The results of the quantitative study, together with data relevant to each animal used, are given in the table.

Phagocytosis of Trypan Blue by the Kupffer Cells of Rats of Different Age Groups

Rat	Age, Days	Weight, Gm.	Amount of 1% Suspension of Trypan Blue Injected Intraperitoneally, Cc.	Kupffer Cells Showing Engulfed Particles in 25 "Oil Immersion" Fields (× 930) of Section
1	6	11.0	0.11	20
2	6	11.5	0.115	1
3	6	11.5	0.115	44
4	6	12.0	0.12	31
5	6	12.5	0.125	0
6	6	11.5	0.23	5
7	6	12.5	0.25	0
8	10	12.0	0.12	0
9	10	12.0	0.12	40
10	10	12.0	0.12	89
11	10	11.5	0.23	102
12	10	12.0	0.24	156
13	15	17.0	0.17	174
14	15	19.0	0.19	112
15	15	19.0	0.38	186
16	18	25.0	0.25	260
17	18	27.0	0.27	280
18	18	24.0	0.48	217
19	18	25.0	0.50	313
20	23	40.0	0.40	268
21	23	50.0	0.50	279
22	23	39.0	0.78	348
23	40	102.0	1.02	268
24	40	105.0	1.05	302
25	40	84.0	1.68	278
26	60	88.0	0.88	264
27	60	87.0	1.74	284

COMMENT

The observations recorded in this paper show clearly that at birth rats are not provided with so effective a means of phagocytosing particles of trypan blue parenterally introduced as they later acquire. This difference has been demonstrated thus far only with the Kupffer cells of the liver, but it appears likely that other phagocytosing cells may show a similar nonspecific difference in function with age, though perhaps in lesser degree. At any rate, since the Kupffer cells have long been shown to be among the most significant cells of the defense mechanism, the demonstration of this difference with them alone goes far toward implicating an improvement in function with age in phagocytic cells as an important force in natural resistance. At the same time, this single defense agency must not be considered solely responsible for natural resistance. The gradually acquired capacity of animals to elaborate antibodies as well as hormones, the changes in the permeability of tissues in general and the alterations in the natural diet as animals grow older, together with differences in genetic constitution and other fundamental differences between animals, must be considered in attempting to explain the natural resistance acquired with maturity.

CONCLUSIONS

There is a difference in capacity for phagocytic function between young and old rats, the Kupffer cells of nursling animals being less able to phagocyptose particles of trypan blue than the Kupffer cells of older animals. This difference can be correlated with a gradually acquired resistance of rats against a natural blood flagellate (Trypanosoma lewisi) of this animal. It is suggested that the resistance which the rat naturally acquires against this parasite as it grows older is in part accounted for by a gradually acquired enhancement in the phagocytic capacity of the host's cells.

ENDAMOEBA HISTOLYTICA

EXPERIMENTAL INFECTION OF THE STOMACHS OF DOGS AND CATS

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The object of this investigation was to induce infection with Endamoeba histolytica in the gastric submucosa of dogs and cats and study the nature of the tissue reaction. The animals were infected by inoculating the submucosa with cultured forms of E. histolytica of human origin. As long as the overlying mucosa remained intact, there was reason to believe that the lesion arising at the seat of inoculation would resemble the basic lesion of amebic disease. But with the disintegration of the mucosa and the consequent exposure of the amebas to the action of the gastric juice, the fate of the trophozoites and the further development of the lesion became a matter of speculation.

It has long been thought that gastric juice exerts a destructive action on the trophozoites of E. histolytica. Councilman and Lafleur [1] reported that E. histolytica exerts no action on the stomach because the conditions there are unsuitable for its multiplication. Ujihara [2] incubated cysts of E. histolytica with gastric juice for twenty-four hours at 37 C. and found that the greater part remained undigested. Penfold, Woodcock and Drew [3] used pepsin in an acid medium as an excysting agent and obtained negative results. Chatton [4] fed cysts to cats and killed the animals after periods varying from three and one-half to seventeen hours; except for the disappearance of the chromidial bars, he found that the cysts passed through the stomach unchanged.

The resistance which the cysts of E. histolytica display toward the action of hydrochloric acid has also been investigated. Yorke and

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1. Councilman, W. T., and Lafleur, H. A.: Johns Hopkins Hosp. Rep. **2**:395, 1891.
2. Ujihara, K.: Ztschr. f. Hyg. u. Infektionskr. **77**:329, 1914.
3. Penfold, W. J.; Woodcock, H. M., and Drew, A. H.: Brit. M. J. **1**:714, 1916.
4. Chatton, E.: Bull. Soc. path. exot. **10**:834, 1917.

Adams [5] exposed cysts to hydrochloric acid in various dilutions and at varying temperatures for thirty minutes. The exposed cysts were then cultured. From cysts exposed to 0.2, 1 and 5 per cent hydrochloric acid at from 20 to 25 C. they obtained positive sixteen hour old cultures of vegetative amebas. Dobell and Laidlaw [6] employed the same acid in a constant dilution at a constant temperature and found that the cysts of E. histolytica readily withstood 0.2 per cent hydrochloric acid for two hours at room temperature and that some cysts hatched after even three hours' exposure.

Hegner [7] believed that the fate of the trophozoite of E. histolytica after exposure to hydrochloric acid might have some bearing on the biologic significance of encystment, and injected trophozoites from carefully prepared cultures of E. histolytica into the stomach of a guinea pig. The animal was killed one hour later, but no amebas were present in the stomach; specimens, alive and moving, were recovered in the small intestine 6, 12, 20, 28, 34, 38, 44 and 51 inches (15, 30.5, 50.5, 71, 86, 96.5, 111.5 and 129.5 cm.) distal to the stomach. From this result Hegner concluded "that trophozoites may pass unharmed through the anterior portion of the alimentary canal and apparently may set up infections in the regions where they are normally localized, but that this probably is not the usual method of infection in nature."

Dobell [8] also studied the effect of acidity on the trophozoites of E. histolytica. He exercised the greatest precaution in obtaining material which contained only unencysted forms. His experimental results indicate that the trophozoites can withstand exposure to 0.18 per cent hydrochloric acid at 37 C. for any time up to one full hour. Dobell stated that "in the acid the amoebae at once become rounded and motionless, and most of them soon appear—and are—dead or dying. It is only by attempting to make cultures from them afterwards that one can determine whether such forms are really dead or alive."

The greatest precaution should be observed in translating the results of these experiments into terms of amebic infection in man. The demonstration of the acid resistance of E. histolytica, particularly in its unencysted forms, raises the question whether the trophozoites live as harmless commensals within the lumen of the stomach or as parasites able at intervals to invade the gastric mucosa. In any consideration of host-parasite relations it is necessary to take into account the resistance of the host as well as the virulence of the parasite. The ability of the

5. Yorke, W., and Adams, A. R. D.: Ann. Trop. Med. **20**:279 and 317, 1926.
6. Dobell, C., and Laidlaw, P. P.: Parasitology **18**:283, 1926.
7. Hegner, R. W.: Am. J. Hyg. **6**:593, 1926.
8. Dobell, C.: Parasitology **19**:288, 1927.

gastric epithelium to resist both the mechanical penetration and the lytic action of E. histolytica is an important factor. But such resistance does not follow immutable standards. The gastric mucosa is constantly being exposed to mechanical and thermal injury, and the normal capacities of its epithelium for resistance against infection may thus be modified. If, nevertheless, the possibility of trauma is omitted from consideration, it may still be open to question whether the hydrochloric acid is able at all times and in all parts of the stomach to prevent an invasion of the normal mucosa by E. histolytica. From a theoretic standpoint it should not matter whether the organism invades the epithelium of the colon or of the stomach except as the location may affect the distribution of the endoparasite; once past this epithelial barrier the amebas are in position to initiate a lesion. But the probability of an invasion of the mucosa in the human stomach by E. histolytica is reduced to one of great uncertainty when it is borne in mind that such an involvement would hardly pass unnoticed in the histologic study of gastric lesions.

The human stomach has never been reported as the seat of a primary lesion in amebiasis; it has been mentioned by several investigators as a site for complications. Craig [9] at autopsies examined 60 persons who died of amebic dysentery with special reference to complications and observed that the stomach was chronically inflamed in 1 of almost every 2 examined. The form of gastritis noted came next in frequency to chronic enteritis and was sufficiently severe to be a factor against recovery. Clark [10] studied the reports of postmortem examinations in 186 cases of amebiasis and cites 2 cases in which an abscess of the liver involved the stomach, causing in one case an obstruction and in the other a perforation of the stomach. James [11] reviewed the findings of Clark and also examined the protocols of the observations at autopsy in 29 additional cases of amebiasis. He noted that there is no a priori reason why amebas that have passed the liver should not lodge in any or all of the organs or localities in which the parasite has been claimed to be present.

> But if this were true [said James] some indication of such localization might reasonably be expected at those autopsies, in which all the damage possible appears to have been done. When one sees in a single instance the entire colon a mass of ulcers, from the ileocecal valve to the rectum, the liver riddled with multiple ulcers, without any other evidence of amoebic invasion, one is permitted a certain scepticism as to whether this parasite is often carried to other localities, or is the cause of not infrequent pathological conditions elsewhere.

9. Craig, C. F.: Am. J. M. Sc. **128**:145, 1904.
10. Clark, H. C.: Am. J. Trop. Med. **5**:157, 1925.
11. James, W. M.: Ann. Trop. Med. **22**:201, 1928.

The records of over 20,000 amebic patients and the reports of over 3,000 autopsies on patients with amebiasis were reviewed by Musgrave [12] in his twenty years of tropical experience. The article embodying the results of this extensive clinical study was edited by Reed, who wrote thus concerning complications:

> Stomach disorders are particularly frequent in all tropical countries and naturally are often associated with amoebic disease in a casual manner. However, in addition to this, there is more frequent and undoubtedly mutually dependent association between the two conditions, particularly in the later stages of a prolonged amoebic infection. Gastralgia is particularly frequent and may be present with but mild amoebic infection. The different types of gastralgia with achylia gastrica and hyperchlorhydria are seen and gastric ulcer is relatively more frequent than in other diseases. After eliminating the usual factors, there remains frequent association of the two diseases which is hard to explain without acknowledging some form of interdependence.

It seems that inoculation of the gastric submucosa with cultured forms of human strains of E. histolytica and observations on the development and nature of the resulting lesions might add something to present knowledge of the characteristics of E. histolytica and perhaps throw some light on the causative factors underlying the process of ulceration in the human stomach.

MATERIALS AND METHODS

Three different strains of E. histolytica of human origin, identified separately by the letters C, G and H, were used in these experiments.[13] The amebas were maintained in artificial cultivation on slants of liver infusion–agar overlaid with sterile rice flour.[14] Every forty-eight hours transplants were made from sediment obtained at the bottom of the culture tube by means of a pipet and incubated at 37 C.[15] Every strain grew well in this medium, and the development of the amebas in vitro was consistent for each strain. The material for injection contained the amebas and an accompanying flora of yeasts and bacteria.

The viability of the inoculum was tested prior to inoculation by placing a drop on a glass slide, adding a drop of 5 per cent aqueous solution of eosin and studying the microscopic details; all specimens contained at least from 10 to 15 amebas per field, and in some the organisms were much more numerous. The inoculums employed in two thirds of the experimental animals of the series contained only strain G, those in 5 animals contained only strain H, while those for the rest of the animals consisted of a combination of G with H or of H

12. Musgrave, W. E.: Am. J. Trop. Med. **11**:469, 1931.

13. The strains were obtained in culture from Dr. Bertha Kaplan Spector (deceased), associate protozoologist, United States Public Health Service, Chicago, and research associate in the department of medicine of the Douglas Smith Foundation of the University of Chicago, who originally cultivated them from patients infected with E. histolytica.

14. Cleveland, L. R., and Collier, J.: Am. J. Hyg. **12**:606, 1930.

15. The strains were cultivated in vitro by Miss Virginia Ryan, research bacteriologist in the research laboratories of the Illinois Department of Public Health, Chicago.

with C. The amount of the inoculum injected in each case varied between 0.2 and 4.5 cc. In preparing the larger inoculums the sediment and liquid from several culture tubes were pooled to make up the requisite amounts for inoculation.

After an injection of pentobarbital sodium into the peritoneal cavity, the abdomen of the animal was opened through a high midline incision in the anterior wall. The pyloric end of the stomach was delivered through the wound. The anterior wall of the pyloric antrum or canal was chosen as the site for inoculation. The needle of the syringe was thrust through the serosa and muscularis, whereupon the required amount of inoculum was introduced into the submucosa. The accuracy of introducing the inoculum into the submucosa by such a blind method may be questioned, but the difference in the sense of resistance offered to the penetrating needle by the serosa and muscularis, on the one hand, and the submucosa, on the other, is so great that one is never in doubt regarding the position of the needle point. Moreover, it is almost impossible to enter the pyloric lumen with the needle for the reason that the mucosa bulges in advance of the needle point until it contacts the opposite wall, giving rise to the danger of penetrating the posterior wall rather than to that of entering the lumen. Owing to one accidental inoculation of the abdominal incision, special precaution against such contamination was thereafter strictly observed.

Altogether, 51 dogs and 8 young cats were inoculated. Only animals which looked healthy and well nourished were selected, in order that the results might be based on trustworthy material. The age of the first 4 cats ranged from 3 to 9 months, while that of the last 4 ranged between 7 weeks and 3 months. The first 14 dogs in the series were kept in individual cages. The other dogs were assigned to two fairly large rooms joined by a passage; here the dogs adapted themselves to confinement, played together and were maintained under a strict quarantine. The cats were kept in individual cages in a sunny room with good ventilation.

A milk diet was given during the first three to five days following inoculation and thereafter a general diet consisting of 1 part of fresh ground beef heart and 2 parts of a prepared food in a dry form, composed of cereal, bone, meat and minerals. It was found desirable to kill the animal to be examined in the morning when the stomach was empty. Immediately after death the cardiac and pyloric ends of the stomach were ligated, and the lumen was filled by injection of a 3.5 per cent solution of formaldehyde. The stomach was then removed and placed in a jar containing a solution of formaldehyde of the same strength. A careful autopsy was made on every animal in the series, either when it was killed or as soon as possible after death. Four hours later the stomach was opened along either the lesser or the greater curvature and carefully examined, after which the extent and intensity of the macroscopic lesion were recorded. Later the specimens were cut into small blocks of tissue, embedded in paraffin, sectioned and stained with hematoxylin and eosin; over 30 blocks of tissue were studied in serial section.[16]

OBSERVATIONS

The inoculation of human strains of E. histolytica into the gastric submucosa gave rise to the formation of gross gastric lesions in 36 dogs and 5 cats (69.49 per cent of the series); in the remaining 15 dogs and 3 cats (30.51 per cent) a lesion could not be demonstrated even with the

16. This work was carried out in the technic room of the department of anatomy under the direction of Dr. O. F. Kampmeier.

aid of a hand lens. The lesions were without exception confined to the stomach; the small and large intestine and the liver failed to show any gross evidence of extension. During the course of the experiment 21 dogs and 2 cats died; 6 dogs of this group presented, in addition to the gastric lesion, some intercurrent disease, while the remainder contained only the amebic lesion. At varying intervals after inoculation 30 dogs and 6 cats were killed. The duration of observation varied from one to one hundred and three days, the average being slightly over four days for the animals that died and about thirty-five days for those that were killed. The majority of the animals displayed only a moderate constitutional reaction to the inoculation; their appetite returned on the first or second day following the injection, and diarrhea or loss in weight was usually not observed with the infection. There were a number of animals that died while the amebic process was still in an early stage, 10 within twenty-hours and 8 within four days, and these showed a strong general reaction to the inoculation.

The earliest lesions observed in the series occurred in dogs that died within twenty-four hours. A lesion of such brief duration consisted of a region of hemorrhagic exudation in the submucosa with moderate congestion of the overlying mucosa; the involved layers presented considerable induration but no apparent thickening or elevation. Surrounding the area of exudation in the submucosa was a zone of infiltration consisting of lymphocytes, plasma cells, polymorphonuclear leukocytes and histiocytes. The gastric pits and glands were filled with a cellular débris, and many of the chief cells were detached from the basement membrane. There was marked engorgement of the involved capillaries and vessels. One dog of this group presented an hourglass constriction of the stomach at the level of the lesion, set up by tonic contraction of a segment of circular muscle fibers, and the hemorrhagic exudation in the lesion extended into the submucosa overlying the constriction.

Circumscribed nodules appeared at primary sites in dogs that died within three days after inoculation, and the various forms are displayed in figure 1. The extravasation of serum and red blood corpuscles into the interstices of the submucosa was sufficiently extensive to increase the depth of this layer to two or three times its normal thickness, and peripheral to it was a region of infiltration with numerous polyblasts, plasma cells and polymorphonuclear leukocytes. Many sections contained large depositions of hematin from the hemoglobin set free by disintegration of the red corpuscles. In some lesions the hemorrhagic exudation extended into the connective tissue septums of the inner part of the muscular coat, causing swelling of the septums and converting the adjoining smooth muscle tissue into a homogeneous hyaline material. The mucosa at the apex of the nodule was transformed into a mass

of granular detritus, while at the periphery the outline of the deeper part of the glands was still visible. The muscularis mucosae was surprisingly well preserved in sections of many of the lesions. The vessels were congested or thrombosed. An occasional displaced parietal

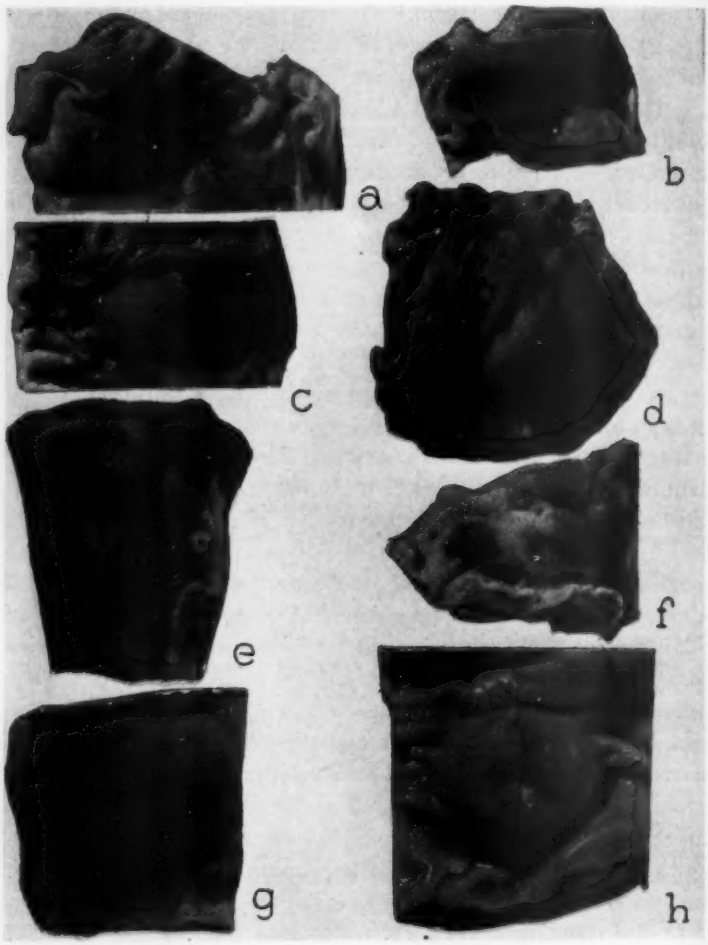

Fig. 1.—A group of large circumscribed nodules, varying in age from forty-eight to seventy-two hours, occurring at sites of inoculation. The mucosa covering these specimens was deeply congested, with early softening. (*f*) The mucosa was in part disintegrated. (*h*) The submucosa was converted into a soft, moist mass of gray necrotic material, which gave rise to the umbilication in the central part of the intact mucosa.

cell of a fundus gland was found to bear some resemblance to an ameba, especially when surrounded by a clear space, and then caution was necessary to make the proper differentiation.

The soft nodular lesion portrayed in figure 1 *h* occurred in a dog that lived only three days; the involved submucosa was reduced to a moist, slightly coherent mass, which was perhaps responsible for the umbilication marking the center of the intact mucosa. The liquefied mass in section showed a dense cellular infiltrate in its central part and a fibrinous exudate at the periphery, in which the inflammatory cells were less dense; the muscularis mucosae remained unbroken.

Nodules arose also at secondary sites, and most of these developed as an extension of the process in older specimens. They occurred as single and multiple lesions with considerable variation in size and shape, as shown in figure 2. The specimens exhibited in *a* and *b* came from dogs killed eighty-nine and one hundred and three days after inoculation, in which the pyloric mucosa, even after the most careful inspection, failed to reveal a primary lesion; the mucosa covering each lesion presented an apical ulceration, while the serosa contained an eccentric sloughing area of pinhead size. The multiple lesions in *c* and *d* came from a dog that died within thirty-six hours after injection; there were thirty or more small nodules distributed in a definitely linear manner over the entire gastric mucosa on, as well as between, the folds.

The overlying mucosa in most of these secondary nodules was hyperemic, and at the apex of the lesion it contained a minute ulcerated area of pinhead size or larger, from which a sinuous tract led downward to a cavity of flasklike profile, filled with a hemorrhagic exudate or a brown gelatinous débris. The cavity was usually located in the deeper part of the mucosa, but in several instances it occupied the submucosa and the sinus leading to it and then perforated the muscularis mucosae. A few parietal cells were seen in the débris either in the tract or in the cavity, the staining qualities of which were well preserved. Except for the region embracing its proximal end, the sinuous tract was surrounded by tissue in which there was little, if any, cellular inflammatory reaction. In one lesion there occurred a small, irregularly shaped cavity in the mucosa which failed to present a communication with either the surface or a contiguous cavity.

Ulceration became manifest as early as the third day after inoculation, and the destructive phases of this process continued to be predominant in some of the primary lesions for as long as eighteen days. The ulcers in figure 3 indicate the various stages of development in these lesions of primary origin. In many of them the cavity communicated with the surface through a wide opening, but this was not true of all the lesions. Each of the three ulcers in line in the specimen shown in *c* presented a slitlike opening on the surface, which was continuous with a piriform excavation below. This specimen came from a dog killed on the ninth day on account of a protrusion of the greater omentum through the

lower half of the abdominal wound; the most distal of the three ulcers extended to within 0.5 cm. of the pyloric sphincter.

The crater of the primary ulcer usually extended down through the mucosa and submucosa; in the more susceptible animals it involved a

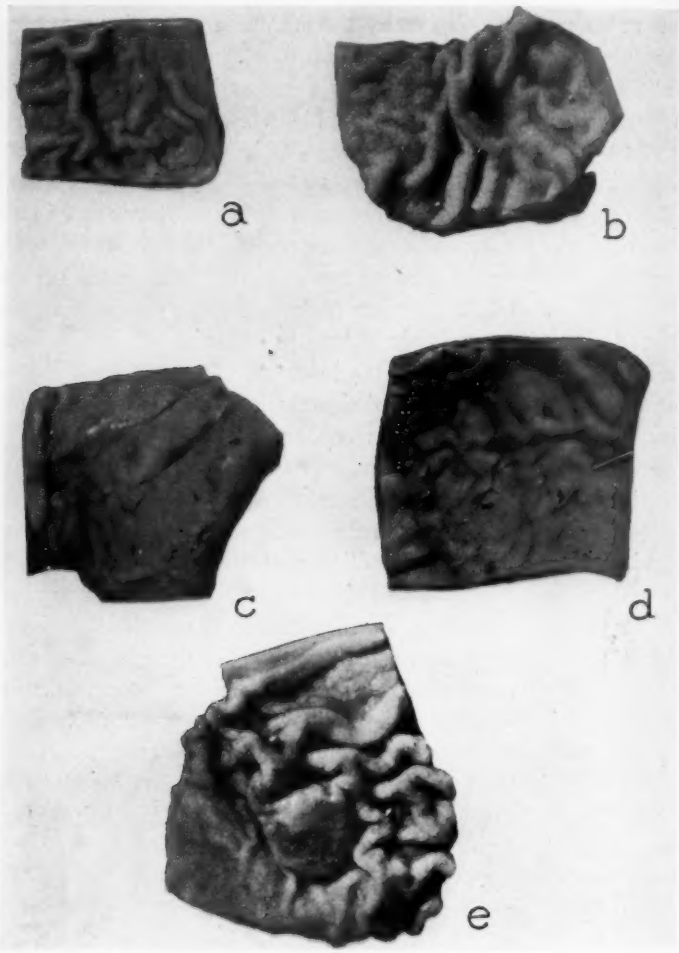

Fig. 2.—A group of nodular lesions occurring at secondary sites, many of them presenting minute apical ulcerations: (*a* and *b*) specimens taken from dogs eighty-nine and one hundred and three days after inoculation; (*c* and *d*) multiple superficial lesions in a dog which died within twenty-four hours after inoculation; (*e*) a lesion located within 5 cm. of the cardiac sphincter and associated with many small superficial erosions as shown in figure 4 *d*.

large part of the muscularis. An extensive gangrenous process took place in a lesion of eighteen days (*c* in fig. 4) in which the floor was composed only of thickened serosa. In the early lesions the crater was

filled with necrotic material, only partially separated from the base and sides. The spread of the amebic process was rapid and extensive in the submucous layer, where the advancing infiltration and softening of the tissue led to undermining of the mucosa above. The mucosa in the central

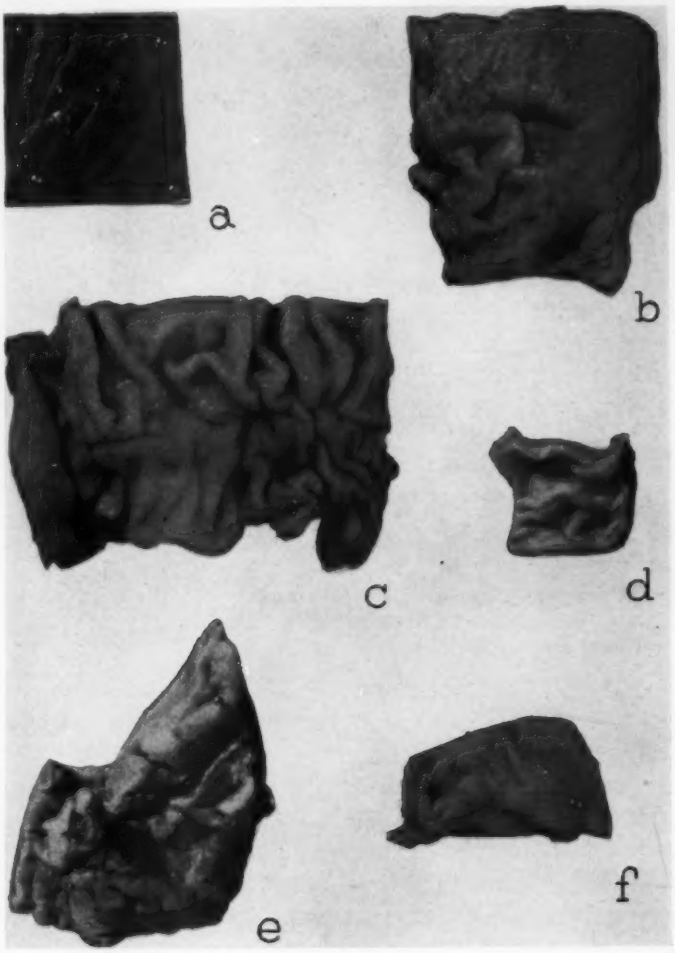

Fig. 3.—A group of ulcers appearing at primary sites: (*a*) lesion with punched-out appearance; (*b*) induration and undermining of margins; (*c*) three button-hole ulcers in line in a dog killed after nine days; (*e* and *f*) excavations filled with grayish brown necrotic material, which was firmly adherent to the floor and sides.

portion was cut off from its nutrition and cast off as a slough, and with further destruction in the submucosa the undermined mucosa at the edge of the ulcer became everted. Two inflammatory zones could sometimes

be distinguished in the transition from normal tissue to the completely necrotic area. The proximal zone was marked by a serous exudate with numerous red blood corpuscles and the peripheral zone by an extensive infiltration of polyblasts, plasma cells and polymorphonuclear leukocytes,

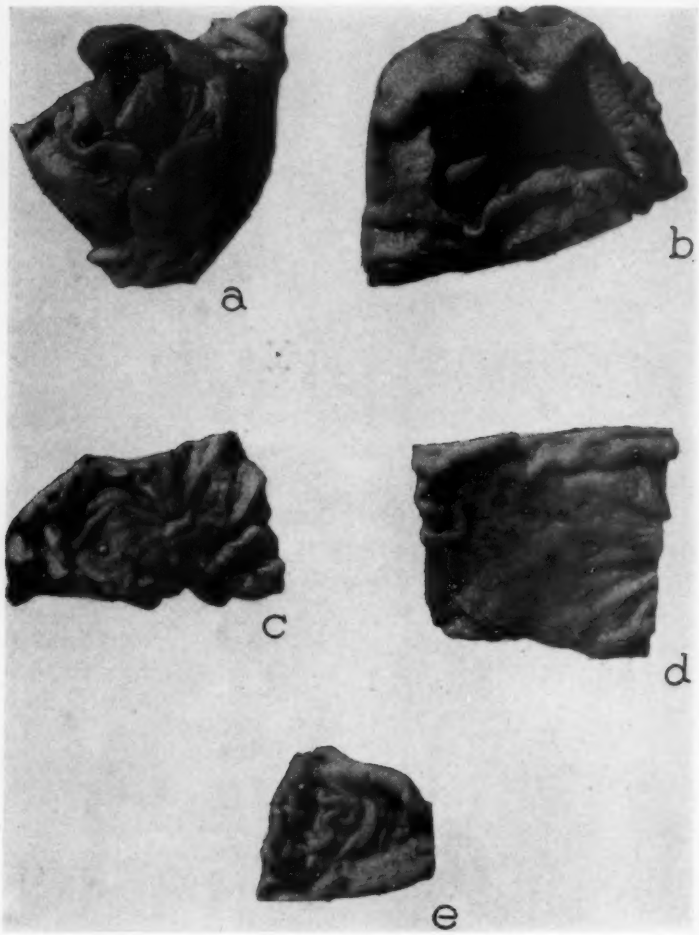

Fig. 4.—Larger gangrenous ulcers are illustrated in *a* to *c;* the lesion in *c* is eighteen days old and the thin, almost translucent floor is composed of only a thickened serosa; *d* and *e* show multiple superficial erosions.

of which cells the last predominated when cocci and bacilli were present in the lesion. The submucosa was greatly thickened in the inflammatory area, which caused an elevation of the edge of the ulcer. Large ulcerative processes were observed in 8 dogs and 4 cats, in which the necrotic

material was cast off, with the formation of an excavation of variable size and shape, and the floor was covered usually with an adherent grayish brown material; sections often revealed two layers in the floor, a superficial stratum of fibrin with a rich cellular infiltration and a deeper layer of numerous fibroblasts and newly formed capillaries. The vessels in the wall of the ulcer were congested or thrombosed and occasionally presented obliterating endarteritis; the walls of vessels were usually edematous and infiltrated with inflammatory cells, and the resistance which they displayed to the lytic action of E. histolytica was at times striking.

Amebas were found in sections of only two specimens in the series. The first was a large gangrenous ulcer appearing at the site of inoculation in a cat which died three days after injection and in which the postmortem examination was delayed about twelve hours. The amebas, which were lodged in the wall of the lesion, contained granular cytoplasm, surrounded by the characteristic clear space, and a nucleus which was spherical and somewhat inconspicuous (fig. 5 *A-C*). The second specimen was a primary ulcer occurring in a dog killed four days after inoculation, in which the tissues were fixed immediately after death (fig. 6 *A* and *B*). The amebas were present in the fibrin of the floor of the ulcer; the nuclear structure, owing perhaps to the increased intensity of the stain, was clearly visible, particularly the layer of fine chromatin granules lining the achromatic nuclear membrane internally and the karyosome with the clear zone surrounding it. Secondary infection occurred in only three ulcerated lesions, in which there was great destruction of tissue and in which the fixation was delayed from eight to twelve hours; the floor and sides of these three ulcers were uneven and ragged, containing numerous cocci and bacilli, with a predominance of polymorphonuclear leukocytes.

Ulcerated lesions, like the nodular lesions, occurred also at secondary sites. The age of these lesions was determinable only from the stage of their development. Multiple superficial erosions were observed in two specimens (fig. 4 *d* and *e*), one from a dog and the other from a cat, killed, respectively, after twenty-one and thirty-five days. The erosions in the dog occurred without a demonstrable primary lesion, while those in the cat were associated with a large primary sloughing ulcer. The lesions were distributed over the whole stomach, and in the specimen from the dog there was also a large nodule with partial mucosal disintegration close to the cardiac sphincter; an occasional buttonhole ulcer occurred among the erosions in both specimens. Each erosion consisted of an area of disintegration involving the gastric glands, around which

was usually a dense cellular infiltration. The secondary lesions associated with the older lesions are described in connection with healed lesions.

The destructive and inflammatory processes in ulceration were gradually replaced by regeneration in the various tissues, and phases

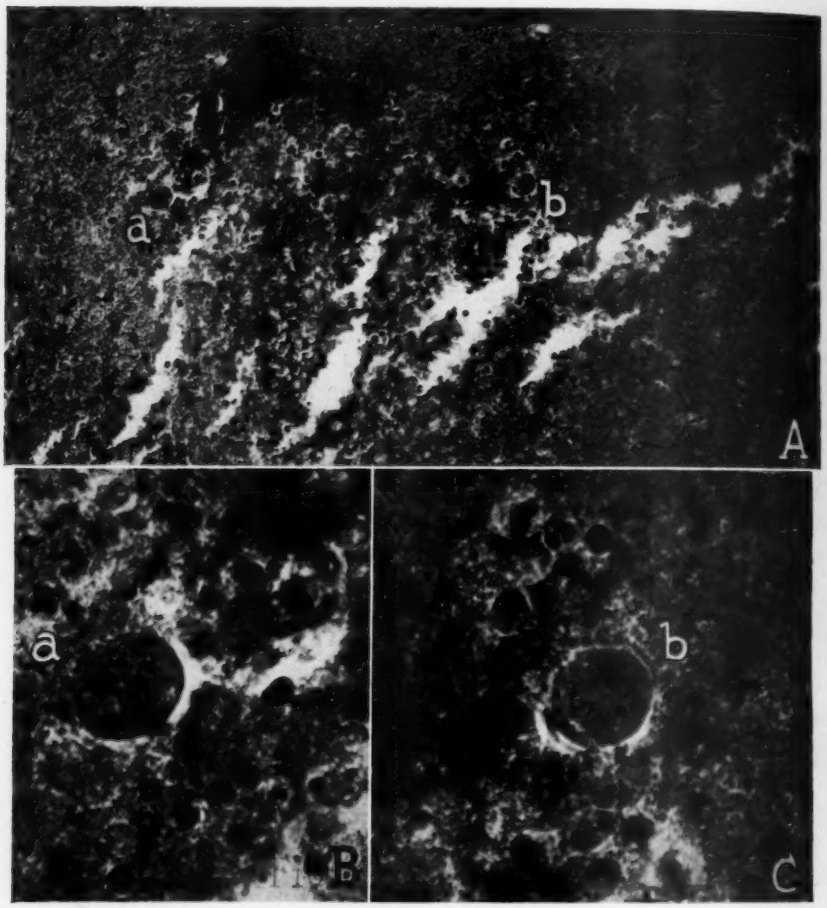

Fig. 5.—*A*, section (× 90) of the side of the ulcer displayed in figure 3*f*, in which the submucosa contains two amebas (*a* and *b*); *B* and *C*, the same section showing the amebas under higher power (× 800).

of all these processes were occasionally seen in one and the same specimen. The epithelium regenerating from the surface and from the gastric glands appeared as early as the fourth day as flattened cells lining the cavity of the ulcer, and at about the same time the first signs of organization became evident in the fibrin of the floor. The earliest

lesion to give the gross appearance of being completely healed occurred in a dog killed after fourteen days (fig. 7 *a*), but on histologic study a small part of the floor was found to be still uncovered with epithelium.

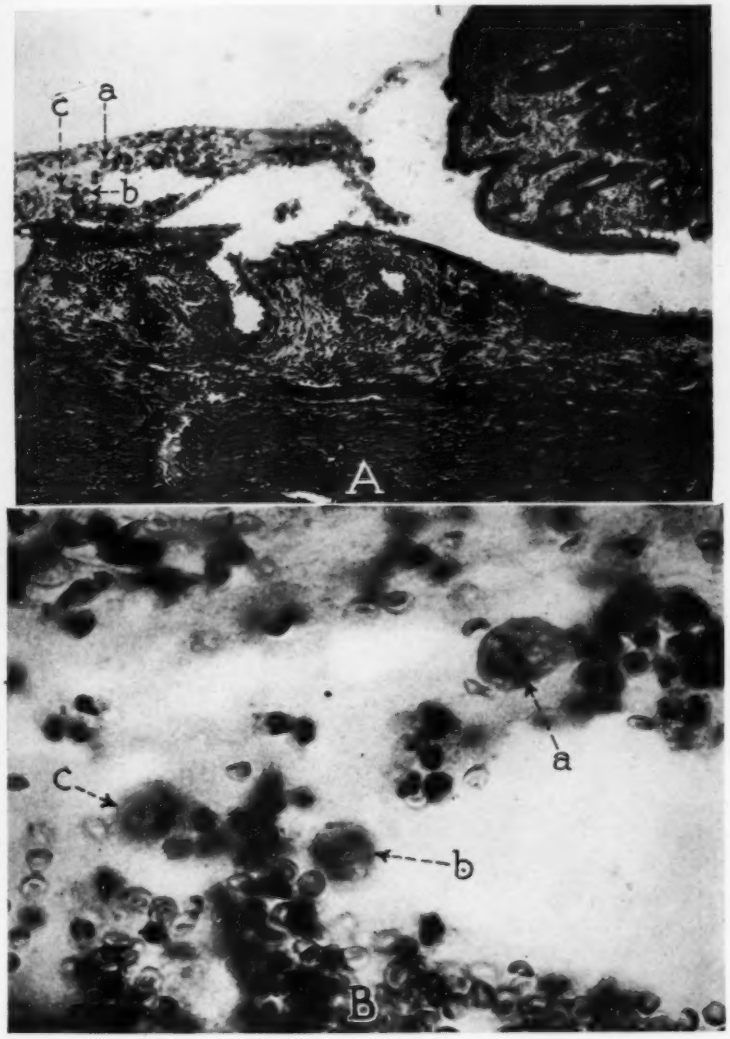

Fig. 6.—*A*, section (× 91) of the ulcer shown in figure 3 *a*, in which several amebas are embedded in the fibrinous floor; *B*, the same section under higher power (× 700), exhibiting at *a* the nuclear structure in one of the amebas; the chromatic granules lining the achromatic nuclear membrane are visible; the two amebas, *b* and *c*, to the left and below are out of focus and do not show their nuclei distinctly.

Healed lesions were found in 8 dogs and 1 cat of the series, the duration ranging from sixteen to ninety-eight days (fig. 7 *a-h*).

The epithelial hyperplasia presented an interesting aspect. As the new epithelium grew out over the granulation tissue in the floor of the

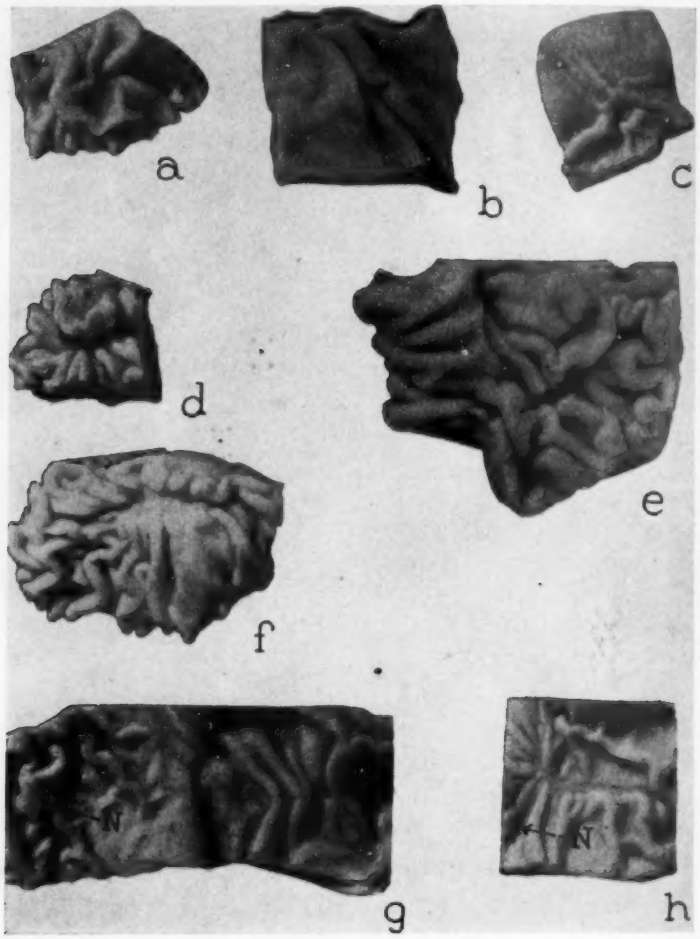

Fig. 7.—A group of healed primary lesions from sixteen to ninety-eight days old; the scars in *g* and *h* are associated with active secondary nodules at *N*, the latter containing a minute apical ulceration.

ulcer, the latter became heaped up by the advancing epithelial edge from each side into an irregular projecting mass, which was reduced only after it became covered with the newly formed epithelium. The cells in the single layer of epithelium in the floor gradually changed from an atypical flattened to a columnar shape; from this layer invaginations

developed later in a downward direction, giving rise to shallow pits and glands with chief cells. The organization in the fibrin of the floor took place in the presence of numerous infiltrating lymphoid cells, histiocytes and leukocytes. Isolated strains of muscle fibers grew up into the organizing tissue of the floor, forming a scant network of muscle tissue at this level.

As healing approached completion, the gastric pits became deeper and the glands more convoluted, and below the bases of the glands there appeared occasionally a newly formed lymphoid follicle; up to the end of the period of observation the glands remained less tortuous than the normal, and the number of glands per unit area was less than that of the normal pyloric mucosa. No regeneration of the muscularis mucosae was observed in any of the specimens. The base of the healed ulcer was usually pigmented and depressed, and the edges were puckered, elevated and everted; the eversion in some lesions was so marked that the edges approximated one another and hid the floor from view. The edge of the old ulcer was always identified histologically by following the muscularis mucosae to its eversion and fixation in the new fibrous tissue.

The completion of the healing process in the primary lesion was not found to signify the end of the amebic process in the wall of the stomach. Three healed lesions were each accompanied by a recent secondary lesion; two of these are displayed in figure 7 *g* and *h*. The first, twenty-three days old, presented two linear scars separated by a normal fold of mucosa, and about 4 cm. proximal to the scars was an active nodular thickening, covered with congested mucosa. The second specimen, sixty-three days old, contained a well healed irregular scar, and less than 1 cm. from its proximal end occurred a nodule with a minute apical ulceration, which led down through a sinus to a hemorrhagic region in the submucosa. In the third specimen (fig. 2 *a*), which was eighty-nine days old, there was no evidence of a primary lesion, but on the anterior surface of the corpus, close to the antrum and the greater curvature, the mucosa presented an active buttonhole ulcer with a hemorrhagic area in the submucosa.

COMMENT

The inoculations led to the production of a large number of gross lesions as seen at autopsy. A wide variety of lesions was obtained in which to study the evolution of the amebic process in the stomach. Many phases of development from the acute to the completely healed stage were seen, and in the active lesions occurred numerous forms of transition from erosions of the most superficial kind to excavations of a deep and extensive nature. The earliest reaction to the inoculation consisted of a hemorrhagic exudation into the submucosa, which became so extensive that the involved layer, as well as the mucosa above, was deprived of

its blood supply. The necrosis which followed in the devitalized mucosa was in part due to the action of the gastric juice; this action was later responsible for the conversion of the hemoglobin into acid hematin, the formation of which caused the black discoloration in the tissues of the floor and sides of the excavation. The infectious process set up by the inoculations in the more susceptible animals became widespread, manifesting extensive lateral involvement and spreading to all layers of the gastric wall. The destructive process differed, however, from the processes produced by other types of experimental infection of the stomach, particularly in the dog, in that it failed to heal promptly; in some primary lesions it remained active for as long as eighteen days, and if the lesions appearing at secondary sites are included in the consideration, the duration of activity exceeded three months. Signs of healing were apparent, of course, as early as the fourth day in the less susceptible animals, and the earliest completely healed lesion was obtained on the sixteenth day after inoculation.

The histologic study of the lesions revealed the unexpected fact that the extent of necrosis in the stomach was out of all proportion to the number of amebas found in the tissues. Most of the sections failed to show amebas. In the sections from only two lesions were amebas identified; the one specimen occurred in a dog and the other in a cat, and about 10 to 15 amebas were found in the serial sections of each. No cause can be cited for the disappearance of the amebas from the gastric lesions. Hara [17] made a study of the disappearance of amebas within the intestines of cats and found that the amebas were greatly affected within an hour after death of the infected animal and that after two hours the great majority of them were almost ready to disappear. Hiyeda and Suzuki [18] observed that the time of disappearance was longer than that quoted by Hara and concluded that the amebas disintegrate in the tissues as a result of postmortem degeneration.

The possibility exists that the gastric juice exercises a destructive action on the amebas after death of the host. The specimens containing amebas in our series were fixed at different intervals, the one from the dog immediately after death and the one from the cat about twelve hours after death. In the sections from the specimen fixed immediately after death the surface epithelium between the gastric pits was in an excellent state of preservation, and the same might have been expected of the amebas unless amebas are more sensitive to the action of gastric juice.

From the experimental results it follows that E. histolytica was able to survive in its vegetative form in the base and sides of each of two

17. Hara, S., cited by Hiyeda and Suzuki.[18]

18. Hiyeda, K., and Suzuki, M.: Am. J. Hyg. **15**:809, 1932.

lesions, unaffected by the gastric juice during the life of the host. This characteristic of the amebas in resisting the action of hydrochloric acid seems almost incontestible, because amebas with typical structural arrangement in the nucleus and cytoplasm were recovered three and four days after inoculation. The experiment by Dobell, which demonstrated the resistance of the trophozoites of E. histolytica to acid in vitro, finds corroboration in our experiments in vivo. Critical data with which to explain the phenomenon of resistance to acid in E. histolytica were not obtainable. It is possible that the cultured forms colonized and reproduced in the looser, more vascular tissue of the submucosa and by an adaptation to the antagonistic conditions of the stomach acquired an immunity which enabled them to continue the invasive process even in the presence of gastric juice. Different types of trophozoites are recognized by Hegner, Johnson and Stabler,[19] but Arnold [20] stated that he knew of no instance in which parasites underwent changes in virulency due to nutritional influences and emphasized that all investigations point toward changes in the natural defensive powers of the host.

The part which the amebas took in the production of lesions in our experiments should be considered. The association of amebas with only two of the lesions in the series may not be sufficient proof that the relation between E. histolytica and the primary lesions is one of cause and effect. Further evidence, however, of this causative relation is found in the close resemblance which all the primary lesions displayed, whether with or without amebas, to the typical amebic lesion of the intestine. The role played by the secondary invaders in the development of amebic lesions is perhaps not as great in the stomach as it appears to be in the intestine. The deterring action of gastric juice against all forms of bacteria is well known, and it is a significant fact that the cocci and bacilli found in the sections of our series occurred only in specimens in which fixation was delayed three hours or longer after death.

The development of lesions at secondary foci, with all the characteristics of amebic lesions, constituted one of the most interesting phenomena of the experiment. Early secondary lesions occurred in close proximity to the primary site as long as three months after the initial inoculation. Though the ultimate course of the primary lesion was unquestionably toward cicatrization, with final restoration of the mucous membrane, it is apparent that the infectious process set up by the inoculations did not become extinct within a period of three months; the presence, moreover, of a healed lesion did not indicate a restitutio ad

19. Hegner, R. W.; Johnson, C. M., and Stabler, R. M.: Am. J. Hyg. **15**:394, 1932.

20. Arnold, L.: Am. J. Digest. Dis. & Nutrition **1**:351, 1934.

integrum, because of the occurrence of secondary lesions. At this juncture it may be of interest to consider the pathway pursued by the infectious process in its distribution from primary to secondary sites. Most of the secondary lesions were either nodular formations situated close to the surface in the mucosa or excavations communicating with the surface by means of sinuous tracts. These structural features indicate that the spread of the infectious agent was along the surface. One specimen, however, contained a small cavity in the mucosa which communicated neither with the surface nor with any adjacent lesion. But this apparently buried cavity is no evidence against surface dissemination, because it is quite possible that the infectious agent might perforate the surface or the wall of a gastric gland without leaving the slightest trace of its path.

No tunnel-like communications were seen between the multiple lesions at secondary sites, nor did these lesions display any tendency toward coalescence. The absence of any inflammatory reaction in the tissues surrounding the sinuous tract or the excavation may be significant in explaining the mechanism of extension of the lesion; indeed, the failure in the formation of a fibrinous exudate or in cellular infiltration may predispose to a perforation of the gastric wall.

That any essential dependence may exist between E. histolytica and the lesions formed at secondary sites may be questioned on the ground that none of the sections of these lesions contained amebas. In this connection it should be emphasized that the spontaneous occurrence of acute or chronic ulceration in healthy dogs and cats, if it appears at all, is rare. Turck [21] reported that in necropsies on 189 healthy and 82 diseased dogs peptic ulceration was not found once. Mann [22] did not find a single lesion of the gastric mucosa in more than 200 practically normal dogs and cats examined post mortem. Ivy [23] with 900 healthy dogs found an acute gastric ulcer in a single dog (0.09 per cent), while petechial hemorrhages and superficial hemorrhagic erosions occurred, respectively, in 4.1 and 2.68 per cent of his series. The great morphologic similarity between the basic lesion of amebic disease and the secondary lesions in our series supports the theory of amebic origin of the latter. The activity of the amebas during the period between inoculation and the occurrence of the secondary lesions becomes a matter of interest if an amebic origin is assumed. In this interval it is quite possible that an equilibrium may exist between the vitality of the amebas and the defense of the host, and the appearance of a secondary lesion may indicate a breaking down of this equilibrium and the beginning of the invasive process.

21. Turck, F. B.: J. A. M. A. **46**:1853, 1906.
22. Mann, F. C.: J. Exper. Med. **23**:203, 1916.
23. Ivy, A. C.: Arch. Int. Med. **25**:6, 1920.

A considerable number of animals failed to show any gross lesion at autopsy. The failure in the development of a reaction after inoculation may be due to changes in either the amebas or the host. The microscopic study of the inoculum preceding inoculation did not reveal any morphologic changes in the cultured forms. There was variation in the motility of the amebas, but no relationship could be established between this variation and the intensity of the reaction in the host. The virulency of the amebas failed to show any change during the five months of cultivation; in fact, the largest and most extensive ulcer occurred in a dog inoculated at the very end of the series.

The resistance which the tissues of the host possess toward the toxic action and the penetrative ability of E. histolytica must be recognized. Unfortunately, little is known concerning the nature of the reaction in the unsusceptible animal. Kessel [24] expressed the belief that the resistance of the host is a more important factor than the difference in the racial virulence of the parasite, and Craig [25] held that a natural immunity exists in some persons. There is a likelihood that the local immunity against the histolytic process is sufficiently effective to allow the formation of only very minute lesions, which heal so rapidly and promptly that no gross evidence of a tissue reaction is discernible. The procedure of inoculating 2 and sometimes 3 animals in succession on the same day from the same culture tube afforded an opportunity of studying the variation in resistance of hosts while the virulence of the parasites remained relatively constant. Three cats of approximately the same age and weight were inoculated in this manner and killed nine, sixteen and thirty-five days after inoculation; a buttonhole ulcer occurred at the site of inoculation in the first, no visible lesion in the second and a number of superficial erosions in the third.

The fact that the stomach of the dog and of the cat has been demonstrated to be experimentally infectible with E. histolytica renders it less certain that the human stomach always escapes invasion on exposure to amebic infection. It is claimed that E. histolytica is the only tissue-invading ameba which has been found in the digestive tract of man. Arnold,[26] studying the bacterial flora of the gastrointestinal tract, observed that under certain conditions the stomach loses the power of controlling the bacterial life within its lumen. He illustrated the difference in the nature of the bacterial flora of the stomach in the normal condition, in the hypofunctioning state and in the acute gastric upset, and suggested that a similar mechanism may underlie the control of

24. Kessel, J. F.: J. A. M. A. **90**:1089, 1928.

25. Craig, C. F.: Amebiasis and Amebic Dysentery, Springfield, Ill., Charles C. Thomas, Publisher, 1934.

26. Arnold, L.: Am. J. M. Sc. **186**:471, 1933.

parasitic infection. In our series the amebic lesion resembled in many respects the gastric ulcer occurring in man. Some of the manifestations common to both are: the localization of the lesion to the stomach; the predominance of single over multiple lesions, with the absence of any coalescence of the latter; the tendency of the process to extend through all the layers of the gastric wall, with the danger of perforation, and, finally, the marked tendency toward recurrence. These characteristics suggest a relation between E. histolytica and the genesis of gastric ulcer in man.

SUMMARY

In 51 dogs and 8 cats the pyloric submucosa was inoculated with three strains of cultured forms of E. histolytica isolated from man. The inoculations gave rise to gross lesions in 36 dogs and 5 cats. Death occurred in 15 dogs and 2 cats in which no lesions were found other than the experimental gastric lesions. Sections of two specimens in the series showed amebas with a typical structural arrangement of nucleus and cytoplasm, indicating that E. histolytica can exist in its vegetative form in the base and sides of a lesion in the gastric wall, unaffected by the action of gastric juice during the life of the host. Early secondary lesions occurred in close proximity to the primary lesions as long as three months after inoculation.

The many characteristics which the resulting lesions possessed in common with the so-called peptic ulcer of the human stomach suggest the possibility of a causative relation between gastric ulcer in man and E. histolytica.

INTESTINAL LESIONS IN CONGENITAL SYPHILIS

A HISTOLOGIC STUDY, WITH A REPORT OF THREE ADDITIONAL CASES, IN ALL OF WHICH SPIROCHETES WERE IDENTIFIED

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Involvement of the intestine in congenital syphilis is distinctly unusual. Even in 1900, when the incidence of frank syphilitic lesions was considerably more frequent than it is at the present time, Oberndorfer [1] was able to collect from the literature only 24 cases, to which he added 1 instance of his own. Fraenkel [2] found only 3 cases in 19,000 autopsies. Among 8,856 consecutive postmortem records from the Charity Hospital of Louisiana at New Orleans reports of only 3 cases were found. These constituted 1.3 per cent of the 230 cases of syphilis in infants who came to autopsy in that institution during the period from Jan. 1, 1929, to Jan. 1, 1937.

The frequency with which gastrointestinal syphilis is found in congenitally syphilitic infants varies with different authors and obviously depends on the amount of material available and the care with which each body is examined. Thus Foerster [3] found the typical lesion only once in 36 cases of congenital syphilis, whereas Mracek [4] found it ten times in 200 cases, and Chiari [5] noted it seven times in 111 cases. The statistics of Birch-Hirschfeld,[6] collected in 1875, show an incidence of 12.5 per cent, and Oluf Thomsen's [7] statistics, collected in 1928, show

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1. Oberndorfer, S.: Virchows Arch. f. path. Anat. **159**:179, 1900.

2. Fraenkel, cited by Wile, D.: Arch. Dermat. & Syph. **3**:372, 1921.

3. Foerster, A.: Würzburg. med. Ztschr. **4**:1, 1863.

4. Mracek, F.: Vrtljschr. f. Dermat. **10**:209, 1883.

5. Chiari, cited by Schneider, P.: Verhandl. d. deutsch. path. Gesellsch. **23**: 177, 1928.

6. Birch-Hirschfeld, F. W.: Arch. d. Heilk. **16**:166, 1875.

7. Thomsen, O.: Pathologisch-anatomische Veränderungen über die congenitale Syphilis bei dem Foetus und dem neugeborenen Kind, Copenhagen, Levin & Munksgaard, 1928.

an incidence of 8.3 per cent. The incidence of 1.3 per cent (3 of 230 cases) which we have found is low, but this is not surprising. Intestinal syphilis is practically always associated with more severe visceral lesions, and the frequency of such lesions, according to syphilographers, has been markedly decreased during recent years by the rather universal application of intensive antisyphilitic therapy.

The purpose of this paper is twofold: to put on record the 3 cases of intestinal syphilis observed, in all of which spirochetes were identified, and to describe the gross and microscopic lesions. Our justification for making the report lies in the fact that to date the number of cases on record in which spirochetes have been identified is very small, and in the fact that none of these cases is recorded in either the English or the American literature. Indeed, so far as we have been able to determine, only a single case of intestinal syphilis is on record in this literature. The report of this case, which was made by Yampolsky and Fowler [8] in 1936, concerns a newborn, full term girl who died an hour after birth. Blood from the umbilical cord gave a strongly positive Wassermann reaction, and a roentgenogram of the bones was suggestive of syphilis. Ascites was present, and slight adhesions between two loops of jejunum were noted. The syphilitic lesions were in the upper portion of the jejunum; they did not involve the mucosa and, according to the authors, resembled tubercles. Apparently spirochetes were not searched for. The case was somewhat unusual because of the character of the gross lesion and the absence of other visceral lesions.

In some 50 per cent of the reported cases of intestinal syphilis, the process was observed in stillborn or macerated fetuses. In 27 per cent the children were born alive but death occurred shortly after birth or within twenty-four hours. A few others lived from ten days to three or four weeks. Ljunggren's [9] subject lived to the age of 2 years, and Roth's and Birch-Hirschfeld's each lived to the age of 3 years.

In recent years, since the specific etiologic agent of syphilis has been identified, special stains for spirochetes have been used in the study of the tissues of the syphilitic infants in some reported cases of intestinal syphilis, but the organism has not always been identified. Ku [10] found spirochetes in only 2 of the 4 cases which he reported, though in all 4 cases the lesion itself, as well as the associated visceral involvement, was characteristic. Spirochetes were first identified in the intestine by Versé [11] in 1906. Since then they have been found by Fraenkel,[12]

8. Yampolsky, J., and Fowler, C. D.: J. M. A. Georgia **25**:154, 1936.
9. Ljunggren, A.: Arch. f. Dermat. u. Syph. **2**:141, 317 and 547, 1870.
10. Ku, D. Y.: Virchows Arch. f. path. Anat. **280**:852, 1931.
11. Versé, M.: Med. Klin. **2**:626, 653 and 682, 1906.
12. Fraenkel, E.: München. med. Wchnschr. **54**:156, 1907.

Thomsen,[13] Warstat,[14] Ku [10] (in the 2 cases just referred to) and Kernau.[15] To these cases we add 3 cases, in each of which spirochetes were found in abundance in the intestinal lesions, as well as in the other organs.

In nearly all cases of congenital intestinal syphilis there is associated involvement of one or more other organs. In more than half of the reported cases the liver was involved, and the lungs were involved in slightly less than half. Weber's osteochondritis was noted in half the cases and cutaneous involvement in nearly a third, syphilitic pemphigus being the most prominent lesion. In Roth's patient, the 3 year old child just referred to, the intestinal lesion was associated with encephalitis and periostitis. Periostitis usually occurs considerably later in life, and encephalitis is also uncommon. Another case of interest was reported by Foerster;[3] in this case there was an associated fibrous inflammation of Glisson's capsule, an association, according to Thomsen, which occurs in 11.61 per cent of all cases of congenital syphilis.

Of particular interest is the association of intestinal syphilis and syphilitic lesions of the stomach. In 8 of the 25 cases collected by Oberndorfer [1] this association was noted, and it was also present in 6 of the 15 cases of gastric syphilis, congenital in 7 of them, which he put on record in the same communication. Oberndorfer was impressed with the frequency of the association, as was Brunner,[16] though the latter considered that intestinal syphilis is more frequent than gastric syphilis.

By far the most common lesion in congenital intestinal syphilis is a raised yellow plaquelike band. These bands occur at irregular intervals on the intestinal wall and tend to encircle the bowel completely. They stand out prominently if the bowel is at all distended when the abdomen is opened. In these areas, in over half of the reported cases multiple ulcerations were observed, and perforation was frequent. The latter was noted in Mracek's,[4] Jürgens'[17] and Ku's [10] cases, as well as in a case of our own, and in all it was followed by generalized peritonitis. Even when perforation has not occurred, adhesion of adjacent coils of bowel may occur at points of involvement by a plastic exudate, and peritonitis can be present without perforation. This happened in 2 of our cases, as well as in cases reported by Ku [10] and Fraenkel.[12]

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13. Thomsen, O., cited by Schneider, P.: Verhandl. d. deutsch. path. Gesellsch. **23**:177, 1928.

14. Warstat, G.: Virchows Arch. f. path. Anat. **212**:195, 1913.

15. Kernau, T.: Centralbl. f. allg. Path. u. path. Anat. **64**:5, 1935.

16. Brunner, C.: Tuberkulose, Aktinomycose, Syphilis des Magen-Darmkanals, Stuttgart, Ferdinand Enke, 1907; in Billroth, T., and Lücke, A.: Deutsche Chirurgie, ibid., 1907, no. 46e, p. 333.

17. Jürgens, cited by Ku.[10]

Other types of lesion have also been described. Birch-Hirschfeld reported diffuse thickening of the intestinal wall. Peyer's patches may be so extensively involved that they are visible on gross inspection, and lesions resembling small gummas may also be identified.

Schneider [18] expressed the belief that intestinal syphilis occurs most frequently in the upper portion of the small intestine, and Herxheimer,[19] that the jejunum is the most frequent site. The recorded cases seem to show a slight predilection for the lower part of the ileum and for the ileocecal region. In about a third of the cases the lesions are chiefly in the ileocecal region; in the remaining cases the distribution is about equal in the upper part of the ileum and the lower and upper parts of the jejunum. Localization in the duodenum is not common and when it occurs is most marked about the papilla of Vater. Ku [10] described 2 cases in which both the jejunum and the duodenum were involved. Roth (quoted by Oberndorfer [1]) reported another unusual case in which the process was localized in the transverse colon. In our cases the lesions occurred variously in the lower part of the jejunum and in the ileum, in the upper part of the jejunum and lower part of the ileum, and throughout the small bowel.

From the histologic standpoint syphilitic lesions in the intestinal tract have frequently been described as gummas. In 1913 Aschoff pointed out that true granuloma is rare, and advanced the view that the specific miliary foci in congenital syphilis are chiefly the result of an acute inflammatory reaction such as occurs in abscess formation. Schneider [18] divided the specific foci in congenital syphilis into three categories: miliary necrosis, abscess-like miliary foci and true miliary granuloma.

The histologic lesions consist essentially of necrosis of the mucosa and submucosa plus severe endarteritis. Proliferation of young connective tissue is usually abundant and involves the whole intestinal wall, especially when it is in the diffuse sclerotic form. Abscess-like miliary foci may also be seen, as in all our cases and in those reported by Ku.[10] Herxheimer [20] considered this lesion to be due to an especially heavy infection with spirochetes. Granulation tissue is stimulated, and leukocytes wander in from the periphery. In some areas the mucosa may be intact but may show severe atrophy and replacement by granulation tissue.

Spirochetes, when they are found, are most abundant at the margins of the areas of necrosis. They are also abundant in the vascular walls and in the perivascular area. In Ku's [10] cases they were most frequent in the mucosa and in the perivascular tissue.

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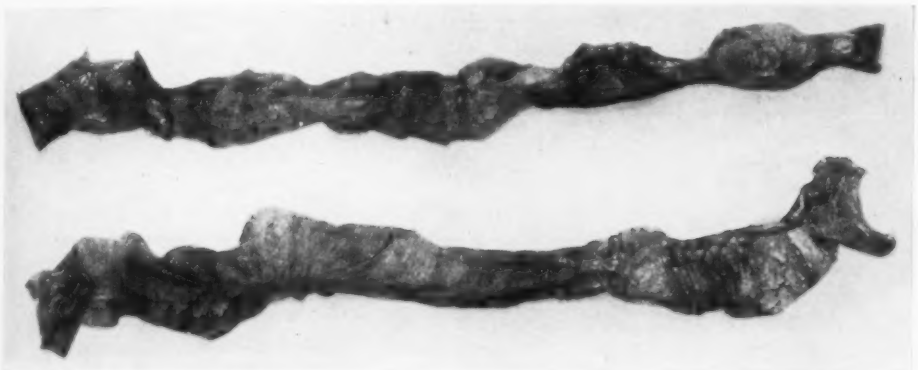
18. Schneider, P.: Verhandl. d. deutsch. path. Gesellsch. **23**:177, 1928.
19. Herxheimer, G.: Ergebn. d. allg. Path. u. path. Anat. **12**:499, 1908.
20. Herxheimer, G.: Verhandl. d. deutsch. path. Gesellsch. **23**:144, 1928.

The 3 cases of congenital intestinal syphilis observed at the Charity Hospital during the eight year period ending in 1937 are presented herewith. Only observations relevant to congenital syphilis in general and intestinal syphilis in particular are recorded.

REPORT OF CASES

CASE 1.—The body was that of a premature, stillborn Negro boy, well developed but poorly nourished, measuring 40 cm. in length and weighing 3½ pounds (1,533 Gm.). The skin was slightly macerated. The lungs were firm in consistency, leathery to the touch and grayish pink; they presented no aeration. The liver was markedly enlarged, and its capsule was smooth and glistening. The tibia showed syphilitic osteochondritis.

In the wall of the small intestine were numerous small cheesy purulent areas. The loops of the bowel were adherent to one another, and many of the ulcerated areas had perforated, so that intraintestinal communications were numerous.



1. Photograph of the small intestine in a case of congenital intestinal syphilis. Note the tendency of the plaquelike lesions to encircle the bowel.

The gross diagnosis was congenital syphilis with involvement of the intestine, liver, spleen, lungs and bones. Spirochetes were found in abundance in all the internal organs.

CASE 2.—The body was that of a premature Negro girl who lived for twenty-seven minutes. It was well developed and well nourished. The crown-rump length was 25 cm., and the crown-heel length, 37 cm. It weighed 4½ pounds (2,041 Gm.). There was a slight icteric tinge over the whole body.

The lungs were pale pink, with areas of consolidation. The liver, which weighed 125 Gm., was firm and light brown. The surfaces exposed by cutting were light brown and mottled, and the markings were indistinct. The spleen, which weighed 40 Gm., was dark red. The surfaces exposed by cutting were firm and fibrous. The pancreas was yellow, slightly enlarged, nodular and firm. The surfaces exposed by cutting revealed distinct lobulations. Section through the femur showed distinct syphilitic osteochondritis.

The upper coils of the small intestine were markedly distended, and there were fine adhesions between the cecum, the lower part of the ileum and the parietal peritoneum, which was thickened. Yellow plaquelike elevations, about 0.5 cm. in width, encircled the ileum at intervals of about 4 cm. throughout its length. At least half of the jejunum was similarly involved. There were occasional areas of necrosis, and corresponding areas of the mucosa showed definite ulcerations. The cecal mucosa was thickened. No ulcerations were present in the stomach, duodenum or esophagus.

The gross diagnosis was congenital syphilis with involvement of the intestine, liver, pancreas and bone. All the internal organs showed spirochetes in abundance.

Case 3.—The body was that of a premature Negro boy who lived for three hours. The crown-rump length was 28 cm., and the crown-heel length, 43 cm. The weight was 4 pounds (1,814 Gm.). A slight icteric tinge was present, but there were no gross lesions of the skin. The abdomen was distended, especially in the upper portion.

The left lung weighed 30 Gm., and the right, 35 Gm. Both were light pink and rather firm, with whitish areas throughout. Little air-containing tissue could be seen. The cut surface felt rather firm.

The liver extended to the left, and almost to the anterior superior spine of the ileum. It weighed 165 Gm. and was dark brownish and rather firm. The surfaces exposed by cutting were dark brown, firm and homogeneous, and normal markings were absent. The pancreas, which was approximately normal in size and weight, was slightly nodular and very firm. The surfaces exposed by cutting were also firm and slightly nodular. The bone showed typical syphilitic epiphysitis.

Neither the esophagus nor the stomach revealed any evidence of ulceration or of tumor. The duodenum also was normal except for marked accentuation and outpouching of the papilla of Vater. There were numerous elevated circular thickenings of the upper portion of the jejunum and the ileum, which were approximately from 0.5 to 1.5 cm. in width and from 0.5 to 0.75 cm. in thickness. In some areas these bands encircled the bowel; in others they were most prominent on the antimesenteric border. On the jejunum these lesions were about 1.5 cm. apart. They became less numerous as the ileum was approached and in its first portion were about 10 cm. apart. In the distal part of the ileum they were more numerous and were 2 or 3 cm. apart. Some of these areas exhibited central ulceration, but the majority showed only a thickened plaquelike elevation. The appendix, cecum, transverse colon, sigmoid and rectum revealed no abnormalities.

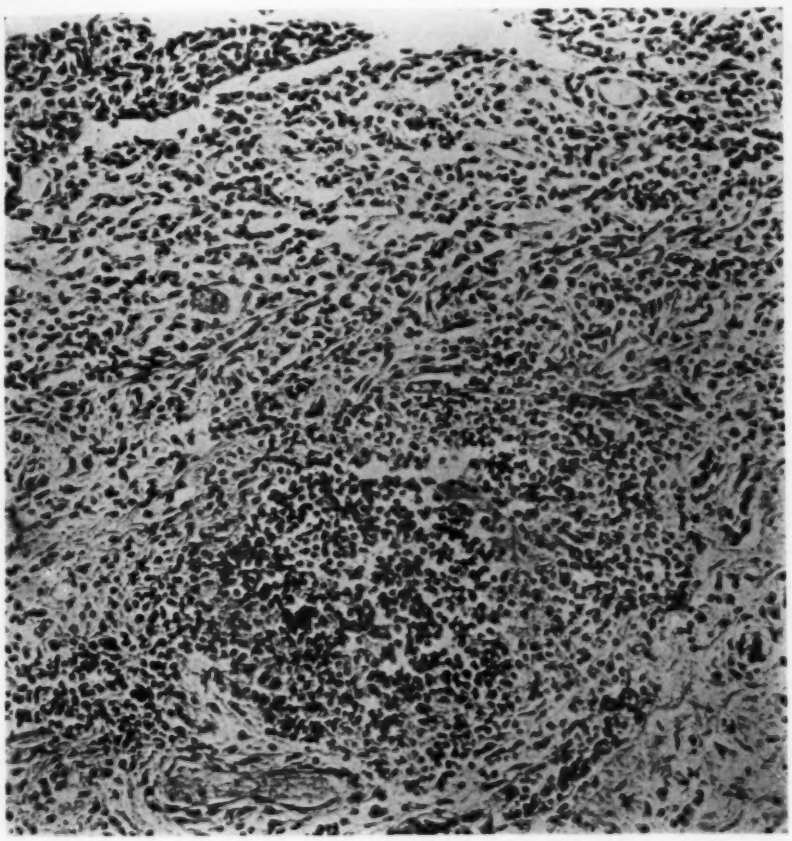
The gross diagnosis was congenital syphilis with involvement of the lung (pneumonia alba), liver, intestine, pancreas and bone. Spirochetes were found in abundance in all the internal organs.

MICROSCOPIC OBSERVATIONS IN CASES 1, 2 AND 3

Since the histologic changes in all 3 cases were practically the same, they will be described jointly, with particular reference to those in the intestine. The other organs revealed the histologic appearances specific for the diagnosis of syphilis. Present in all 3 cases was marked interstitial pancreatic fibrosis—a change mentioned in not more than 15 per cent of the other reported cases. To judge from our observations, this is the most common, definite and reliable pathologic change in congenital syphilis. We have found it in all cases in which there was visceral involvement, and Thomsen [18] stated that it occurred in 86.9 per cent of all cases of congenital syphilis.

The liver, spleen and lungs showed syphilitic involvement in all 3 cases, the lungs exhibiting pneumonia alba in all. All these organs showed spirochetes in abundance.

Sections through the involved areas of the intestinal wall showed absence of mucosa in the ulcerated areas. Both the mucosa and the submucosa showed necrosis of the fibroblastic tissue, only a few well stained plasma cells and endothelial leukocytes being seen. At the periphery this necrotic area merged into an area

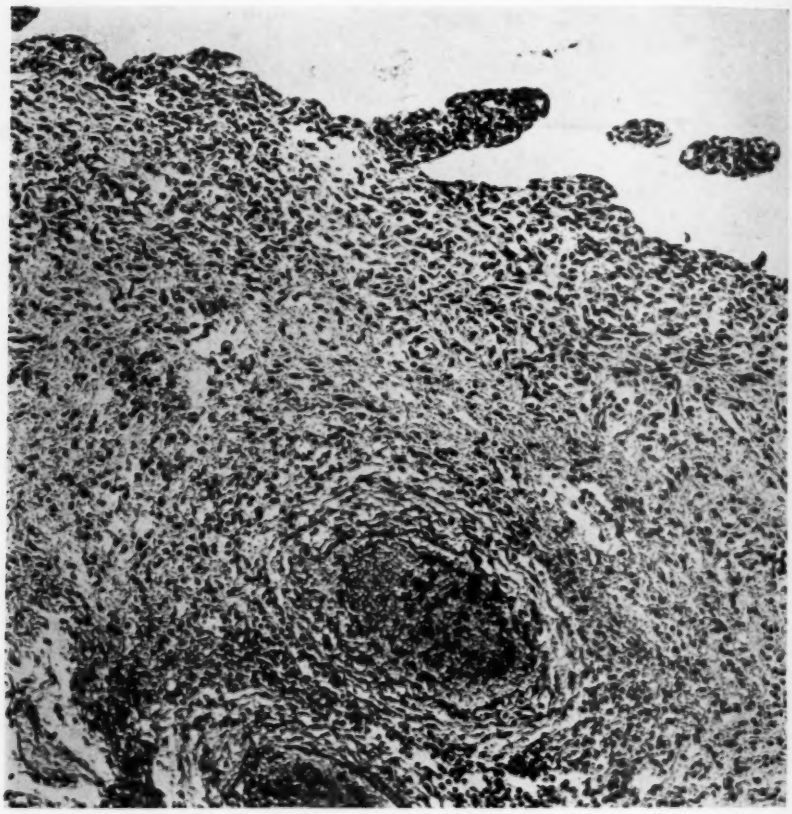


2. Bowel wall showing infiltration of the mucosa and submucosa with plasma cells and fibroblastic tissue. Miliary abscess-like foci, such as can be seen in the lower portion, are an important early finding in intestinal syphilis.

composed chiefly of young fibroblasts and many newly formed capillaries. Within the meshes of this newly formed granulation tissue were many plasma cells and endothelial leukocytes. In some miliary areas of necrosis the granulation tissue was very faint. Some cellular infiltration with leukocytes could be seen. In the area of granulation tissue pyknosis of the nuclei of cells was frequent. The muscular portions, as well as the adventitia, showed many dilated vessels, which were filled with red cells. Some of the red cells had extravasated into the surrounding

tissue and were there broken up into hemosiderin. The hemosiderin, together with the many fat cells present throughout, was probably responsible for the yellowish color. The muscular layers of the intestine were well infiltrated with leukocytes, which showed pyknosis. Plasma cells were present in abundance. In some places the muscular layers had been replaced by young granulation tissue. Endarteritis was marked.

The earlier lesions in intestinal syphilis could be studied best in areas of transition from normal mucosa to more involved portions. Here the plasma cells

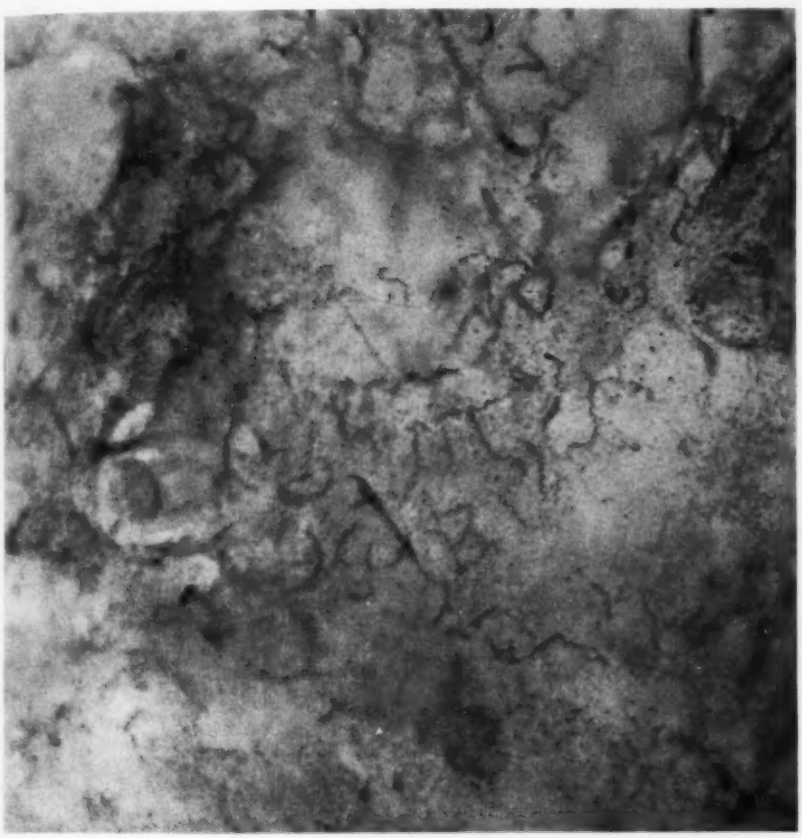


3. Older lesion showing presence of more mature fibroblasts and replacement of the intestinal layers with definite fibrous tissue. Note the beginning fading-out and necrosis of the fibroblasts. Note also the thickening of the wall and the splitting of its layers in the blood vessel.

were numerous, and while the deeper mucosal glands could still be seen they tended to disappear as plasma cells became more numerous and as an early fibroblastic reaction occurred. The mucosa then became transformed into young granulation tissue.

In the submucosa could be seen many miliary syphilomas, corresponding to the kind described by Schneider as "abscess-like" miliary foci. We believe that such lesions represent early stages of the process and that many later become necrotic. Various

combinations of the necrotic and the abscess-like forms were seen. The abscess-like areas were present only when granulation tissue was present, and no giant cells could be seen in any portion. The vessels in the submucosa and in the more normal portions showed extreme dilatation. Little perivascular infiltration could be seen. The newly formed fibroblastic tissue tended to encircle the small vessels in such a manner that on first glance it seemed to be an actual part of the wall of the vessel. The arrangement, however, was apparent and not real; usually there was a small space between this tissue and the arterial wall proper.



4. Section from the wall of the ileum, stained by Levaditi's method, showing numerous spirochetes, some undergoing degeneration (oil immersion).

Spirochetes in abundance were easily demonstrated by silver stains throughout the bowel wall. Degenerated club forms could also be seen. The spirochetes in sections were most numerous around the small vessels and within the walls of vessels. They were present throughout the various tunics of the bowel, even in the necrotic areas, where, however, they were less numerous. They lay in the same planes as the muscle fibers, and the contrast between the longitudinal and the circular distribution was striking.

SUMMARY

Congenital syphilis with intestinal involvement was uncommon even before the era of general specific therapy and is even less frequent at this time. More than 75 per cent of all cases are observed in macerated stillborn infants and infants who live less than twenty-four hours. Some type of visceral involvement is always associated with the intestinal lesion; most usually it is an involvement of the liver or of the lungs. Involvement of bone is also frequently associated with the intestinal lesion.

The most common and most characteristic intestinal lesion is a raised yellow plaquelike band which encircles the bowel. Generalized peritonitis may follow ulceration and perforation of these areas but may also occur in absence of frank rupture. The syphilitic process is chiefly confined to the small intestine; it has a special predilection, from the standpoint of number of lesions and intensity, for the last portion of the ileum.

The lesions include necrosis of the mucosa and submucosa of the involved areas, miliary syphiloma and abscess-like foci. Vascular dilatation, followed later by fibroblastic reaction, probably occurs earliest in the mucosa and submucosa. Subsequent necrosis is frequent. Replacement of the whole bowel wall with fibroblastic tissue is common in the more severely involved portions.

Spirochetes, when they can be identified, are seen in all the layers of the bowel and are especially prominent in the perivascular tissues and in the vessel wall. In the muscular coats they tend to lie in the direction of the muscle fibers.

We have put on record, with a complete histologic study, 3 cases of congenital intestinal syphilis, in all of which spirochetes could be demonstrated.

PATHOLOGIC CHANGES IN THE NERVOUS SYSTEM IN YELLOW FEVER

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In a study of small pieces of brain tissue from 14 persons who died of yellow fever, it was found that there was marked perivascular hemorrhage in various parts of the brain in most of these persons. In view of this finding it was thought desirable to obtain, if possible, the whole brain in cases of this disease in order to determine the localization of the hemorrhages and to study any other change from the normal that might be present in the brain in yellow fever. Accordingly the whole brain was obtained and studied in 20 cases.

METHODS

Sections were made as far as possible through the superior frontal gyrus, the paracentral lobule, various parts of the temporal lobe, the occipital lobe, the cerebellum, the periventricular and subthalamic region at the level of the mamillary bodies, the midbrain, the pons and the medulla, and through the spinal cord in 3 cases. In some cases sections were made also through the lenticular nucleus and the insula. Some of the brains were partly spoiled for study because of postmortem softening, so that the regions studied were not identical in all cases.

Masson's trichome stain was used to demonstrate hemorrhages, and all embedded sections were embedded in pyroxylin. Hematoxylin and eosin stains were also used to demonstrate cellular exudate in the meninges or in the brain. Preparations of myelin sheaths were made in many cases by Loyez'[1] method. Weigert's stain for elastic tissue, with counterstaining by Van Gieson's method, was used in a study of the blood vessels in several cases. Nissl's stain was used for a study of the nerve cells in some cases. Frozen sections were stained for neuroglia by Hortega's method as modified by Globus, also for fat and microglia, in all instances.

GENERAL OBSERVATIONS

After fixation the appearance of these brains seldom varied from the normal. In some instances the pathologist who made the necropsy

From the Department of Pathology, Cornell University Medical College, and New York Hospital.

The pathologic tissues examined in this study and the information regarding the illnesses were obtained through the cooperation of the International Health Division of the Rockefeller Foundation. Tissues and data were supplied from Brazil through Dr. Fred L. Soper, Dr. John E. Elmendorf Jr. and Dr. J. A. Kerr, of the staff of the International Health Division, and Dr. Amadeu Fialho, pathologist of the National Department of Health of Brazil; and from Colombia through Dr. J. H. Paul and Dr. E. R. Rickard of the International Health Division.

1. Loyez, M.: Compt. rend. Soc. de biol. **62**:541, 1910.

stated that the cerebrum and other parts of the brain were congested. Occasionally a few hemorrhagic spots were noted, and in case 6 there was opacity of the cerebrospinal fluid.

Microscopic examination showed fibrous thickening of the arachnoid in several cases, but this was usually normal for the age of the patient. Once or twice slight subarachnoid hemorrhage was encountered.

In all of the cases perivascular hemorrhages were found. Various parts of the temporal lobe and the region at the level of the mamillary bodies were the most frequently affected by the hemorrhages. These hemorrhages were small and usually, although not always, confined to the spaces about small arteries, as well as veins, and often about capillaries. Occasionally a small hemorrhage was noted with no vessel visible from which it was derived. A slight amount of perivascular edema was noted in some of the cases.

There was little evidence of lymphocytic infiltration of the Virchow-Robin spaces or of the meninges. In 9 cases a few infiltrating lymphocytes were seen. No significant loss of myelin was noted in any case. The neuroglia was normal in appearance in all but case 2, and in most of the cases no abnormal amount of fat was seen. The microglia was increased in some cases. The walls of the blood vessels were normal except where hemorrhage had distorted and compressed them. The nerve cells in most of the cases studied varied little from normal, and no inclusion bodies were seen in the cells of the hippocampus or elsewhere.

Much of the hemorrhage in these cases was seen in the sections through the mamillary bodies and optic thalamus. The hemorrhages in this region were usually near the lining of the third ventricle, in the subthalamic region and near the mamillary bodies. Similar hemorrhages were found in this situation in cases of alcoholic encephalopathy, so that this lesion in the subthalamic region is not peculiar to yellow fever. The impression was gained that the lesions in these cases were due to some toxin circulating in the brain and spinal cord in yellow fever. There was little if any evidence of inflammation in the nervous system. There was little suggestion of those changes usually seen in virus infections of the brain.

REPORT OF CASES

CASE 1.—The brain was that of a man aged 62, whose illness lasted seven days and twenty-two hours. At necropsy the meninges were described as slightly congested. There was fibrous arachnoiditis consistent with the age of the patient, with a few red blood cells in the meshes of the pia-arachnoid over the paracentral lobule. The capillaries were congested in this region, but there was no significant edema and no lymphocytic exudate in any of the sections examined. Perivascular hemorrhages were present in the olfactory tract, the paracentral lobule, a site

near the lining of the third ventricle (these were perivenous for the most part), the temporal pole, the pons and the cerebellum. Sections through the cerebellum showed pericapillary and periarterial hemorrhages near the dentate nucleus. The neuroglia, microglia and fat seemed to be normal.

CASE 2.—This brain was from a white man aged 20 years, whose illness lasted five days and twenty-one and a half hours. Hemorrhages were found in the frontal

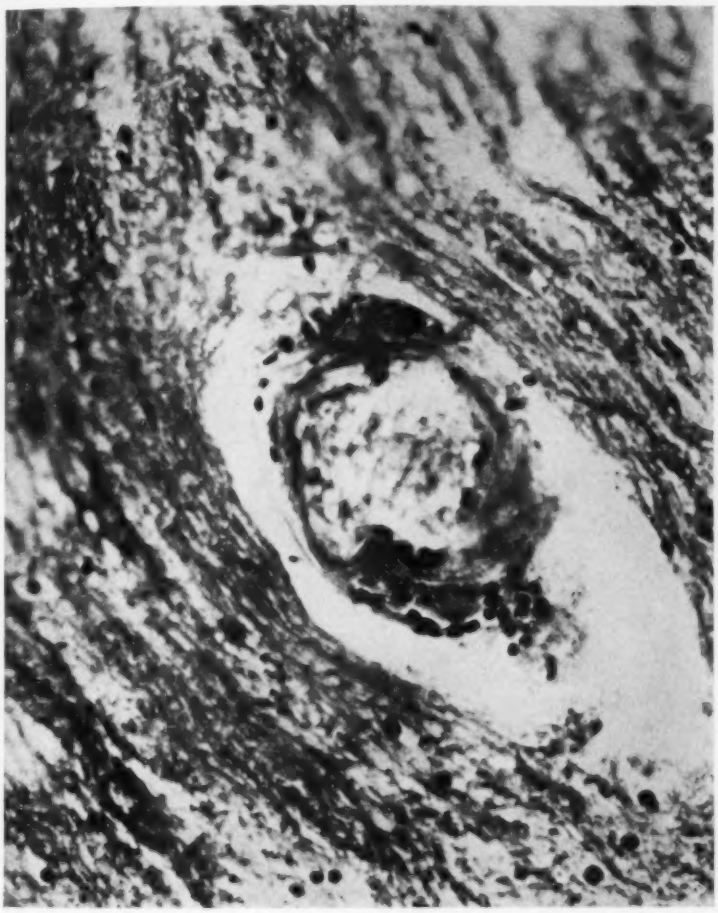

Fig. 1 (case 2).—Section through the pons showing a slight perivascular exudate of lymphocytes and perivascular edema. Hematoxylin and eosin stain.

and temporal poles. The neuroglia, microglia and fat were normal. In the pons there were a few lymphocytes in the perivascular spaces about one or two vessels (fig. 1). There was some edema in the occipital pole and in the paracentral lobule. This brain was rather badly decomposed in places so that only a limited study was possible.

CASE 3.—The brain was from a white man aged 55, whose illness lasted six days and five and a half hours. At necropsy the meninges were described as slightly congested. Perivascular hemorrhages were found in the superior frontal gyrus, the hippocampus, the occipital lobe, the insula, the region at the level of the mamillary bodies, the lenticular nucleus, the midbrain, the pons and the cerebellum. There was a slight subarachnoid hemorrhage over the cerebellum. In the midbrain

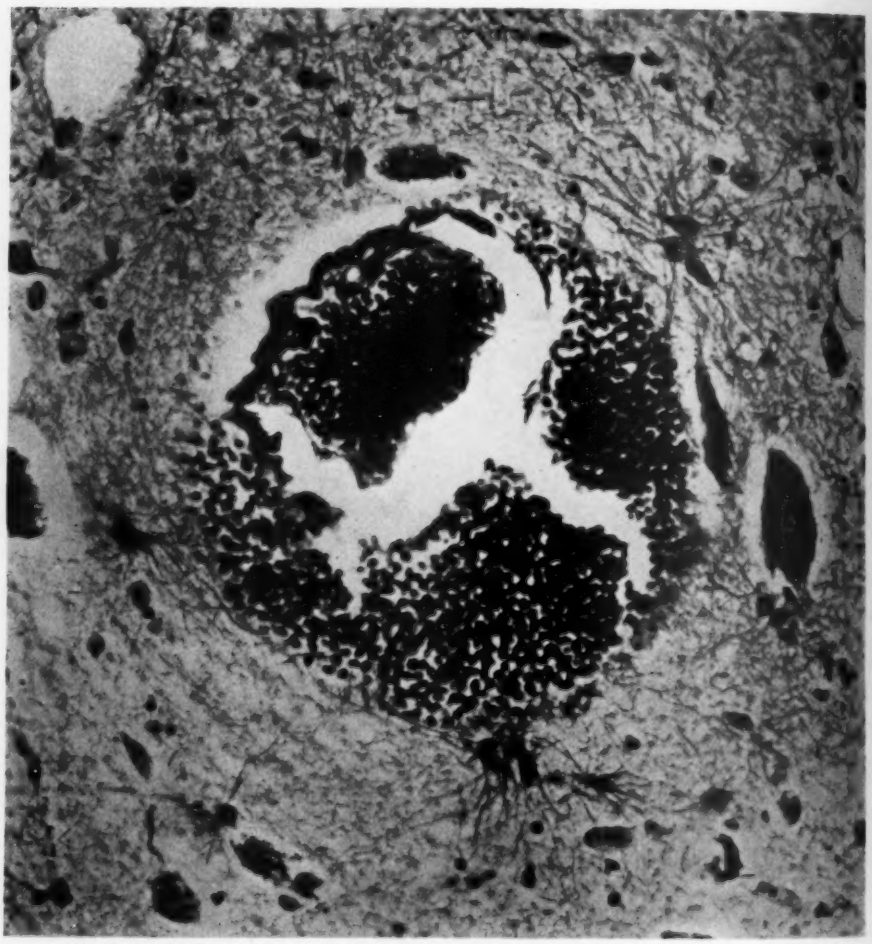

Fig. 2 (case 3).—Frozen section through the pons showing a hemorrhage and at the edge of the hemorrhage some astrocytes in small clusters. Hortega's silver carbonate stain for astrocytes.

a few lymphocytes were present in the adventitia of one or two vessels. Nissl stains of the superior frontal gyrus showed no significant changes. The Weigert stain for elastic tissue with Van Gieson counterstaining showed normal blood vessels. Sections of the superior frontal gyrus stained with Loyez' stain for myelin sheaths showed no loss of myelin. The astrocytes in this brain were increased in number, and figure 2 shows them reacting to the hemorrhage.

CASE 4.—This specimen was from a white man aged 38, whose illness lasted six days and six hours. Perivascular hemorrhages were found in the frontal pole, the superior frontal gyrus, the paracentral lobule, the occipital pole, the region about the third ventricle, the hippocampus, the pons, the medulla and the cerebellum. In the section at the level of the mamillary bodies marked periventricular hemorrhages were present, although none were seen in the mamillary bodies themselves

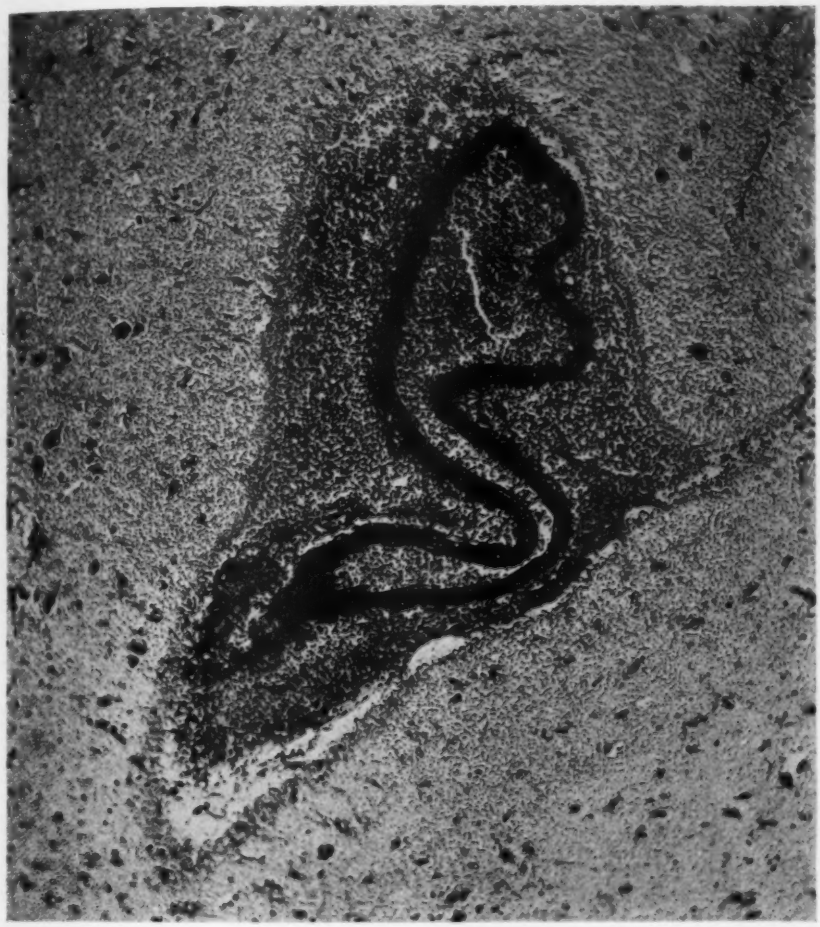

Fig. 3 (case 4).—Section at the level of the mamillary bodies showing a perivenous hemorrhage in the subthalamic region midway between the mamillary body and the massa intermedia. Masson's trichrome stain.

(figs. 3 and 4). There was slight ependymitis in this section, and a few lymphocytes were seen in the adventitia of one or two of the vessels. The astrocytes in the hippocampus and superior frontal gyrus were normal. Nissl stains of the superior frontal gyrus showed no significant change in the nerve cells. Silver carbonate stains of the temporal lobe and superior frontal gyrus showed sclerotic

change in the nerve cells (fig. 5) and an increase in the microglia and fat. Some transitional microglia cells were seen.

Case 5.—This was the brain of a white man aged 36, whose illness lasted six and a half days. Perivascular hemorrhages were found in the frontal pole, the

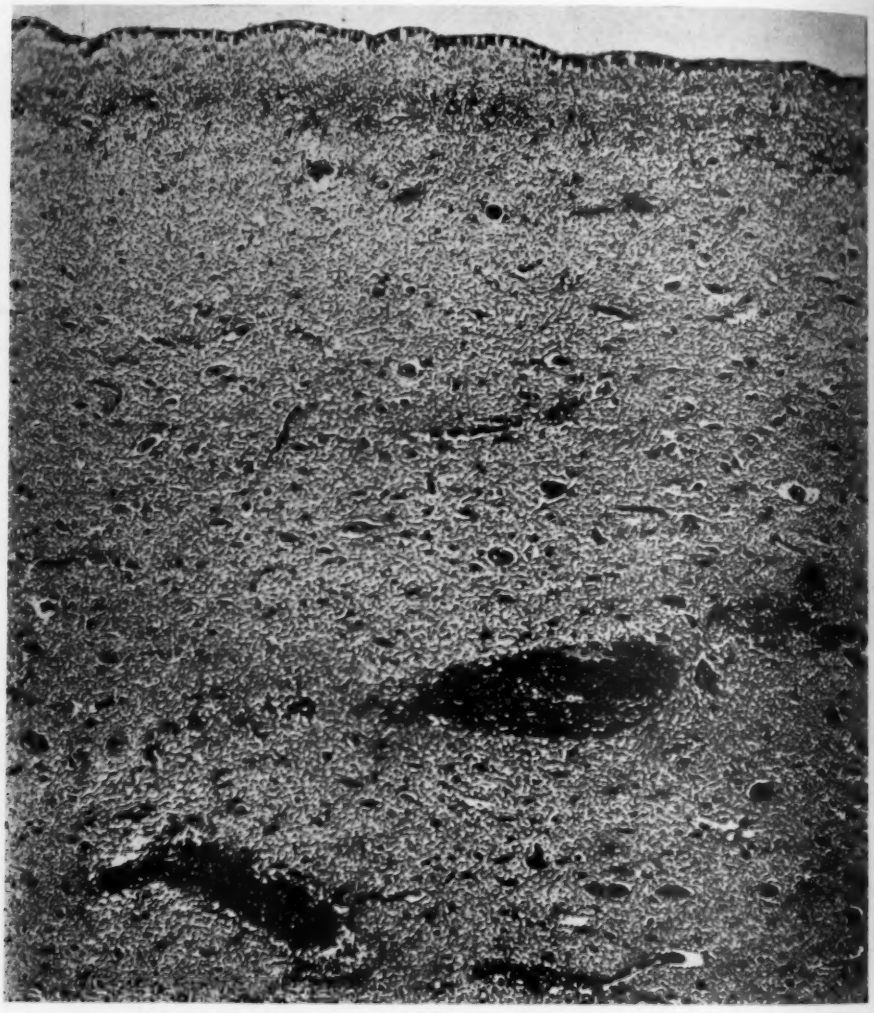

Fig. 4 (case 4).—Hemorrhages in the periventricular region at the level of the mamillary bodies. The ependyma lining the third ventricle can be seen. Masson's trichrome stain.

paracentral lobule and the temporal pole. The astrocytes were normal. The microglia and fat were normal.

Case 6.—The brain was that of a white boy aged 10½ years whose illness lasted six and a half days. Perivascular hemorrhages were found in the frontal pole, the paracentral lobule, the temporal pole, the temporal lobe, the region about

the third ventricle and the medulla. The neuroglia, microglia and fat were normal. There was some fibrous thickening of the arachnoid over the superior frontal gyrus, and perivascular edema was present in this region also.

CASE 7.—The brain was from a white man aged 24, whose illness lasted five days and twenty-one hours. Perivascular hemorrhages were seen in the para-

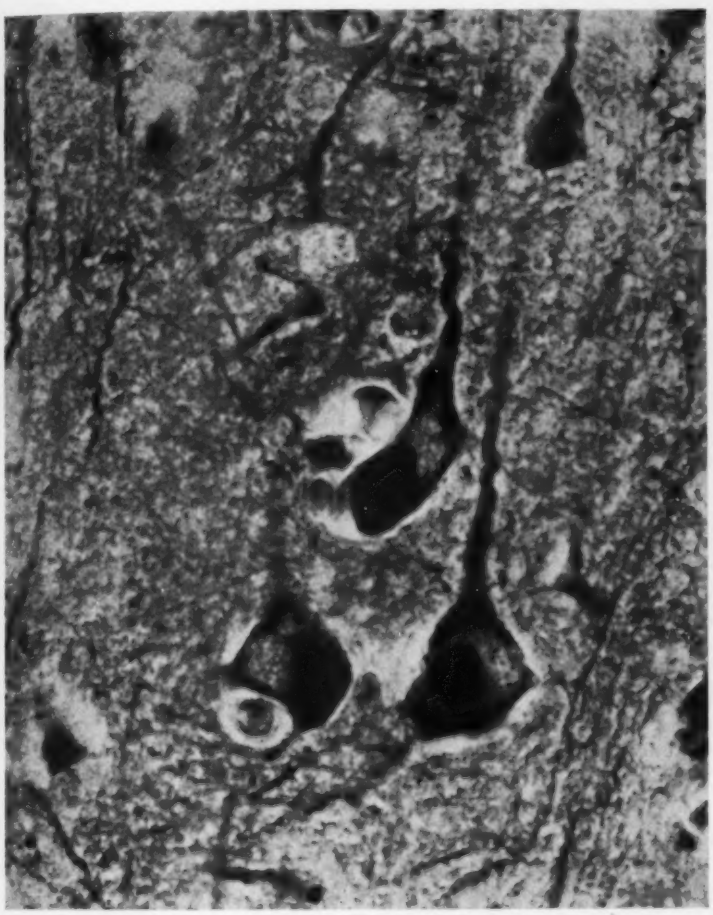

Fig. 5 (case 4).—Frozen section through the superior frontal gyrus showing slight sclerosis of ganglion cells with some increase of fat. Hortega's silver carbonate stain with sudan III.

central lobule, the temporal pole, the region about the third ventricle, the occipital pole and the cerebellum. There was edema in the paracentral lobule. The neuroglia and fat were normal. The microglia was somewhat increased in amount.

CASE 8.—The brain was that of a white man aged 48, whose illness lasted seven days and six hours. At necropsy it was stated that the veins were much dilated

over the cerebrum and that there were small hemorrhagic spots. The cerebellum, pons and medulla were congested. Perivascular hemorrhages were present in the superior frontal gyrus, the paracentral lobule, the temporal lobe, the occipital pole, the tissues about the third ventricle, the midbrain, the medulla, the cerebellum, the

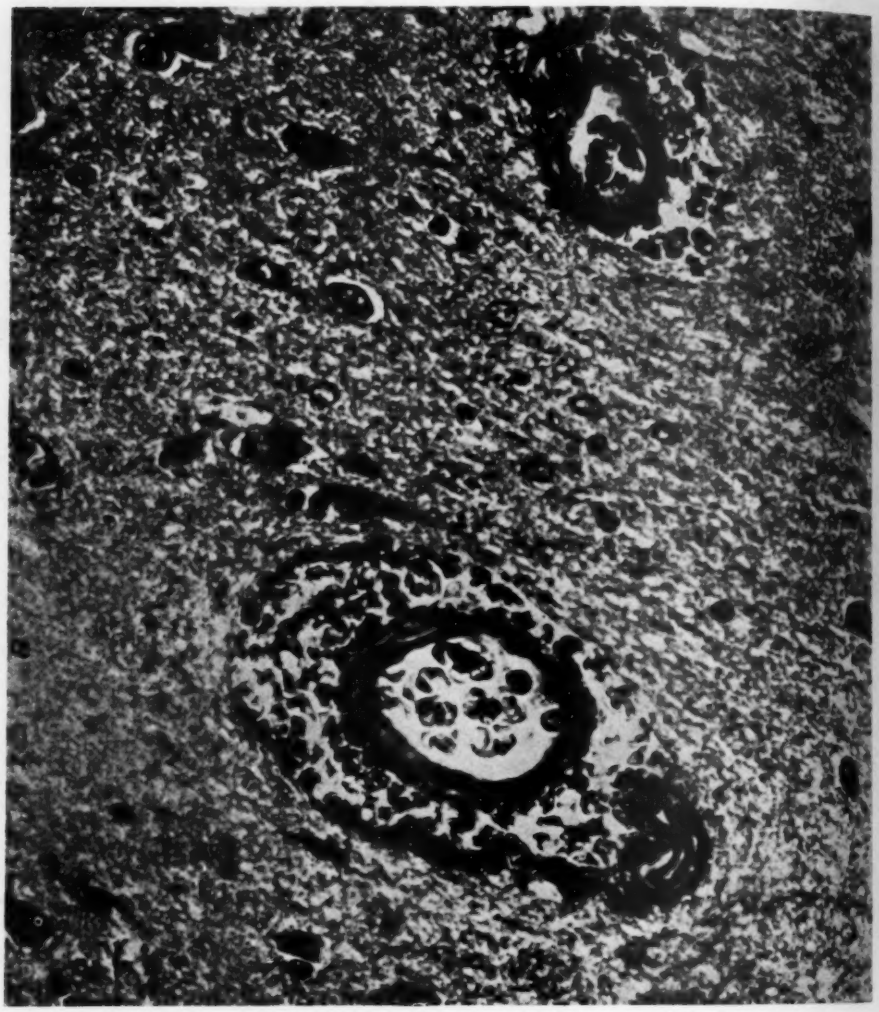

Fig. 6 (case 9).—Section through the hippocampus showing perivascular hemorrhage. Masson's trichrome stain.

olfactory tract and the optic nerve. Weigert's stain for elastic tissue with Van Gieson counterstaining in sections of the superior frontal gyrus showed normal blood vessels. Nissl stains of the superior frontal gyrus showed the nerve cells to be normal. There was some loss of nerve cells in places. Microglia cells were well stained in the temporal lobe and were slightly increased in number; there

were some transitional forms. The fat was increased in amount in the nerve cells of this region and in the perivascular spaces. There was some arteriolar sclerosis in this brain, as well as some fibrous thickening of the arachnoid. There was some subarachnoid hemorrhage over the paracentral lobule, with slight degenerative changes in the nerve cells. The neuroglia in the superior frontal gyrus was normal.

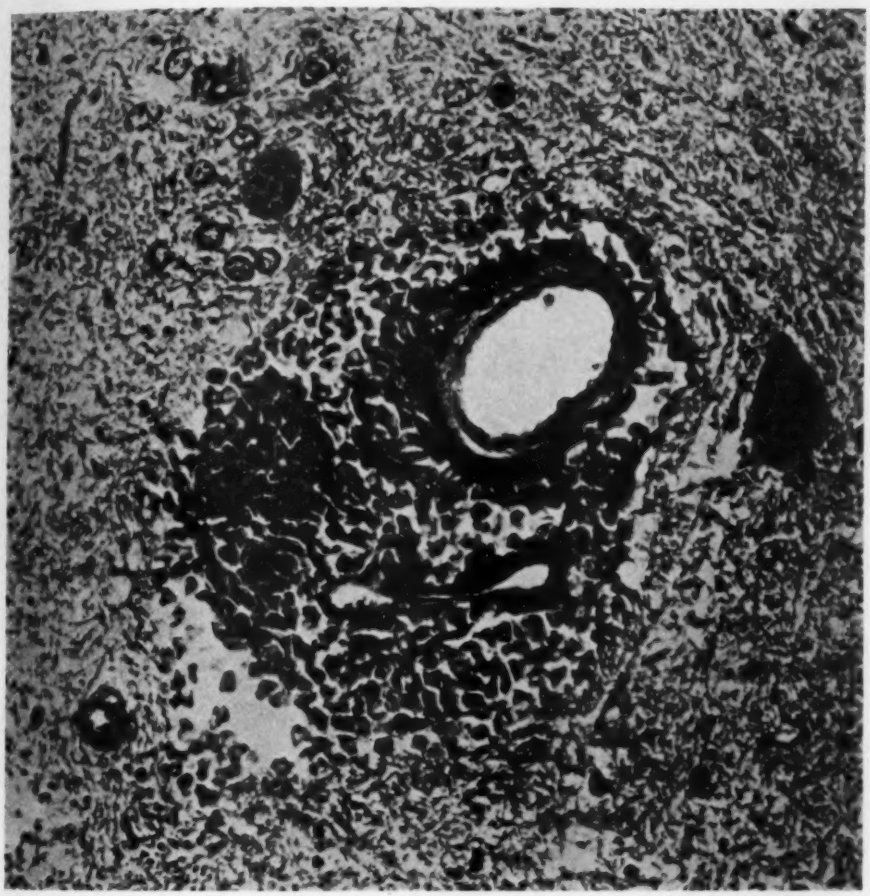

Fig. 7 (case 11).—Section through the lumbar region of the spinal cord showing a perivascular hemorrhage in the gray matter. Masson's trichrome stain.

CASE 9.—This brain was from a white man aged 35, whose illness lasted twelve days. At necropsy the cerebrospinal fluid was said to be opaque, resembling pus. The cerebrum had hemorrhagic spots; likewise, the cerebellum, pons and medulla. Perivascular hemorrhages were found in the subthalamic region, temporal pole, hippocampus (fig. 6), cerebellum, midbrain and occipital lobe. Sections of the paracentral lobule prepared with Loyez' stain showed no loss of myelin. Nissl staining of sections from the paracentral lobule showed no significant changes in

the nerve cells. There was an unusually large amount of fat in the nerve cells of the temporal pole for a man of this age; the microglia cells in this region were normal, although there was some fat about the blood vessels in places. In the temporal pole there was some increase in the subpial neuroglia.

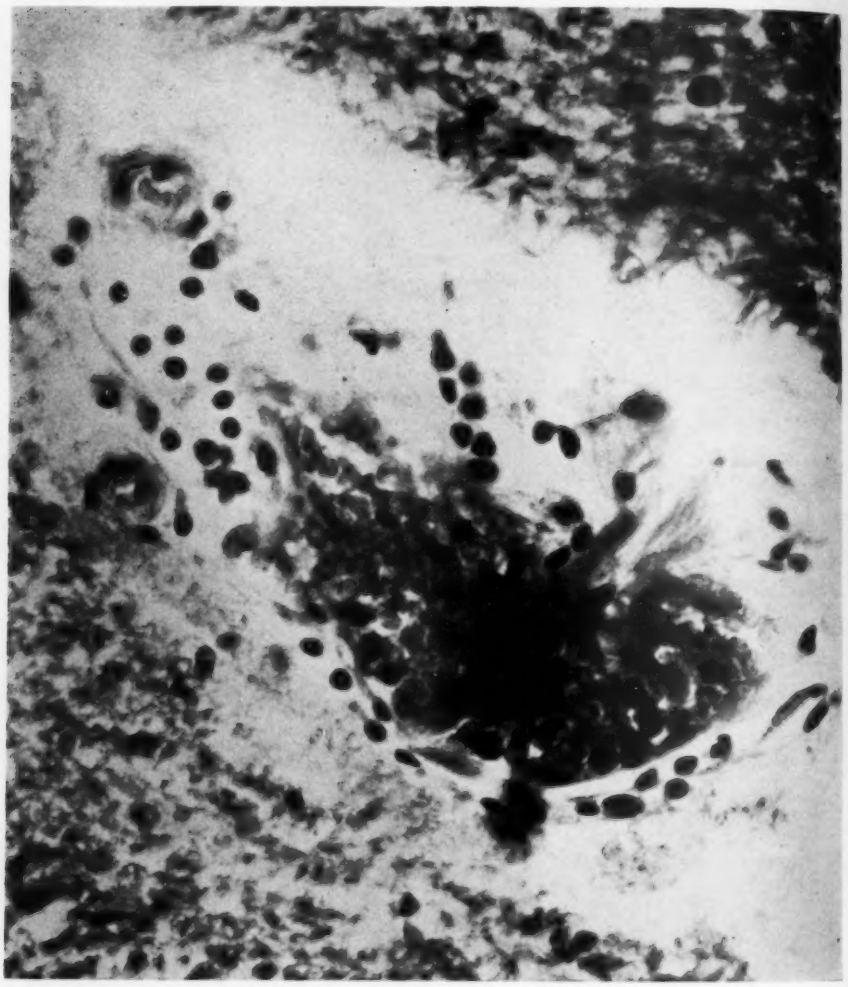

Fig. 8 (case 11).—Section through the lower cervical region of the spinal cord showing some lymphocytes about a blood vessel in the anterior horn of the gray matter and perivascular edema. Hematoxylin and eosin stain.

Case 10.—This was the brain of a white man aged 26, whose illness lasted five days. At necropsy the meninges were reported as apparently normal. The cerebrum, cerebellum and pons were apparently normal. Perivascular hemorrhages were seen in sections from the temporal lobe and subthalamic region. The microglia

was slightly increased in parts of the temporal lobe. The fat in the nerve cells was normal. The neuroglia was normal.

CASE 11.—The brain was that of a white man aged 41. Sections from the temporal pole, the optic thalamus and the midthoracic and lumbar regions of the spinal cord (fig. 7) showed perivascular hemorrhages. There was some perivascular edema, also some pyknosis of nerve cells, in the superior frontal gyrus. The microglia cells were well stained in parts of the temporal pole, with some transitional forms. Fat was normal in amount in the nerve cells. The neuroglia was slightly increased in the white matter of the temporal pole. Sections through the cervical region of the cord showed some perivascular lymphocytes (fig. 8).

CASE 12.—The brain was from a white man aged 27, whose illness lasted four days and two hours. Perivascular hemorrhages were found in the temporal lobe, hippocampus and subthalamic region. The brain was reported to have no unusual appearance at necropsy. An occasional lymphocyte was seen in the adventitia of a blood vessel here and there. The microglia in the temporal lobe was slightly increased in amount, and a few transitional cells were seen. Fat was increased in the cells and about the vessels slightly. The neuroglia was normal.

CASE 13.—This was the brain of a white boy aged 11, whose illness lasted six days. Perivascular hemorrhages were present in the superior frontal gyrus, the temporal pole, the subthalamic region, the cerebellum, the occipital lobe and the olfactory tract. The microglia in the temporal pole stained fairly well and seemed normal. The fat in the nerve cells was normal. The neuroglia in the temporal pole was normal.

CASE 14.—This brain was from a white man aged 20, whose illness lasted four days and ten hours. Perivascular hemorrhages were present in the temporal lobe, the subthalamic region, the pons, the upper cervical region of the spinal cord and the olfactory tract. An occasional lymphocyte was noted in the adventitia of a few vessels in the optic thalamus. The neuroglia, microglia and fat were normal.

CASE 15.—The brain was that of a white girl aged 12, whose illness lasted five days and ten hours. The pathologist who performed necropsy noted that the contents of the cranial cavity appeared normal. There were perivascular hemorrhages in the inferior temporal gyrus, the midbrain, the medulla and the optic nerve. There was some fibrous arachnoiditis over the paracentral lobule; there was some perivascular edema in the sections from the occipital lobe. The neuroglia and fat were normal, but the microglia was increased in amount.

CASE 16.—This specimen was from a white man aged 23, whose illness lasted four days and fifteen hours. Perivascular hemorrhages were present in the temporal pole, the cerebellum and the occipital pole. There were a few lymphocytes in the perivascular spaces of one or two vessels in the temporal pole. Nissl staining of sections from the occipital pole showed no significant changes. Loyez staining of sections from the occipital pole showed no loss of myelin. The neuroglia in the occipital pole was normal, and the microglia in this region was increased. There was no abnormal amount of fat in the cells of the occipital pole. The Weigert stain for elastic tissue with Van Gieson's stain showed normal vessels in the occipital pole.

CASE 17.—The brain was from a woman whose age was not stated. Her illness lasted about six days. Perivascular hemorrhages were seen in the temporal

lobes, the anterior portion of the striate body and the cerebellum. A few perivascular lymphocytes were seen in sections from the midbrain. The neuroglia, microglia and fat were normal.

Case 18.—The brain was that of a boy of 4 or 5 years of age, whose illness lasted about four days. Perivascular hemorrhages were seen in the superior frontal

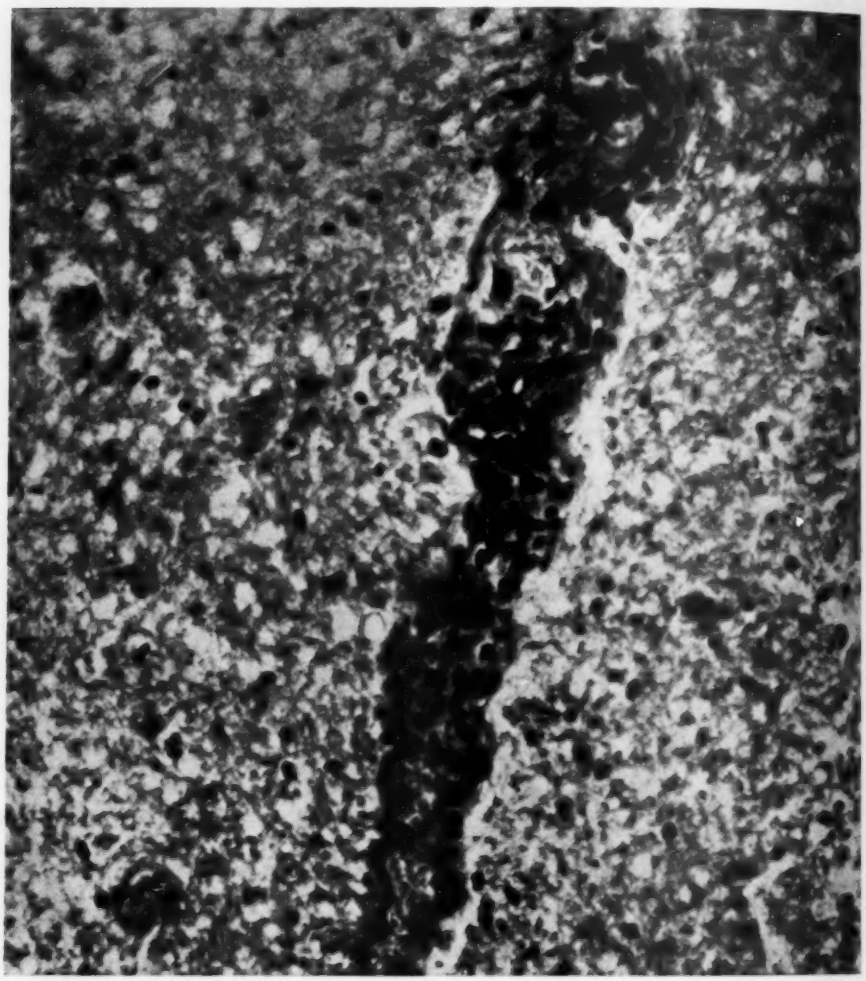

Fig. 9 (case 18).—Section through the optic nerve showing an exudate of lymphocytes about a blood vessel. Hematoxylin and eosin stain.

gyrus, the hippocampus, the midbrain, the subthalamic region, the medulla, the gyrus rectus, the olfactory tract, the optic nerve, the cerebellum and the lenticular nucleus. In the optic nerve there were also a few perivascular lymphocytes (fig. 9). The neuroglia, microglia and fat were normal.

CASE 19.—The brain was from a girl aged 23 months, who died after an illness of four days. Perivascular hemorrhages were present in the hippocampus, the occipital pole, the pons and the temporal lobe. A few perivascular lymphocytes were seen in the hippocampus. The neuroglia was normal. The microglia and fat were slightly increased.

CASE 20.—This brain was from a child of 9, whose illness lasted about four days. A few perivascular hemorrhages were seen in the temporal pole and in the subthalamic region. The neuroglia and fat were normal. The microglia was increased in amount.

In addition to these 20 brains, another was studied in the same way, and although the diagnosis of yellow fever was not confirmed by the findings in the liver, this brain showed lesions exactly like those in the cases in which yellow fever was diagnosed definitely. This brain was from a white man about 35 years of age, who entered the hospital on May 13, 1936, "with a temperature of 36.8 C. (96.9 F.) and a pulse rate of 90 and with the skin and conjunctivae showing jaundice. The patient complained of headache, gastric distress and nausea. Although the patient did not urinate, the bladder was not investigated for the presence of urine. On May 14, the patient presented nervous symptoms, such as picking at the bedclothes. The pupils were dilated. Headache was pronounced. Hemorrhagic vomiting and bleeding from the gums were noted, but neither nosebleed nor melena was reported. Involuntary urination occurred, but material for examination was not collected. The temperature was subnormal on this day; the pulse was not registered. These symptoms continued on May 16, the day of death." Dr. Fred L. Soper, of Rio de Janeiro, Brazil, furnished these notes on the case after observing the patient in the hospital. He noted in addition that this case seemed to be "typical of severe yellow fever with some involvement of the central nervous system, indicated by mental haziness." Dr. Edgar Cruz, who performed autopsy, stated: "On the basis of the observations at autopsy, I made a diagnosis of yellow fever." Dr. Soper stated that the conditions noted at autopsy included jaundice, remains of black vomit in the buccal cavity and melena in the intestines. In the description of the liver by Dr. Laemmert, however, no evidence of yellow fever was noted. Dr. Soper, in discussing this case in a letter to Dr. E. L. Opie, dated March 2, 1938, stated: This case opens up once more the entire question of the validity of a diagnosis of yellow fever from histologic examination of tissues of the liver. It is known that rhesus monkeys sometimes die of yellow fever without showing what are considered to be the characteristic lesions of yellow fever in the liver. There is, however, available in the municipal laboratory of Rio de Janeiro material from more than 125,000 livers from different parts of South America, mostly Brazil. The collection of this material has been made, as you know, through viscerotomy for the purpose of discovering otherwise undiagnosed outbreaks of yellow fever. Practically all cases in which the liver has been thought to show positive signs or changes arousing suspicion in the laboratory have been investigated in the field, with additional laboratory control, when possible, through attempts to isolate the virus and through determinations of immunity in convalescent persons suspected of having had yellow fever. Likewise, during the same period the laboratory examined a fairly large number of livers from persons whose records describe their condition as suspected of being yellow fever in the field but diagnosed as not that disease after examination of the liver. Whenever possible, these cases have likewise been investigated in the field. As a result of this accumulated experience, my colleagues and I have come to have a high opinion of the examination of liver as a method of detecting otherwise undiagnosable yellow fever. However, we have come to realize that yellow

fever is not obliged to follow the rules laid down for it by any one scientist or group of scientists. For example, there is the possibility, no doubt, that the lesion in the liver is quickly obliterated by regenerative processes, so that persons who die from secondary infections or other causes later than the twelfth or fourteenth day may well fail to obtain a positive diagnosis in the laboratory. Likewise, we have come to recognize that the diagnosis of the lesion in the liver is a subjective process and is not capable of the same objective demonstration as is, for example, the finding of the parasite of malaria in the blood smear. We are not in a position to affirm that yellow fever always produces certain lesions in the liver or to affirm that nothing else can produce the lesions produced by yellow fever. Nevertheless, we continue to perform viscerotomy on a large scale and believe it to be by far the most sensitive indicator of the presence or absence of yellow fever in a given region at the time the investigation is carried out."

The brain in this case showed perivascular hemorrhages in the frontal pole, the tip of the temporal lobe, the midbrain, the cerebellum, the subthalamic region and the medulla. There was perivascular edema in the occipital pole. Sections of the frontal pole treated with the Weigert stain for elastic tissue and counterstained with the Van Gieson stain showed normal vessels. The microglia and neuroglia in the temporal pole were normal. Sections of the frontal pole prepared with the Loyez stain showed no loss of myelin. There was some fat in the perivascular spaces, as well as considerable fat in the nerve cells, in sections from the temporal pole. Nissl staining of sections from the frontal pole showed normal nerve cells. My associates and I considered this to be one of the most typical of the cases of encephalopathy produced by yellow fever before we learned that study of the liver did not confirm the clinical diagnosis.

A table is appended to show the sites of hemorrhages in the 20 brains. The table also shows what other regions of the brain were examined in each case in which no hemorrhages were found. As I have noted in an earlier part of this paper, the hemorrhagic condition in the brain and spinal cord in yellow fever resembles very much what my associates and I and what others have seen in alcoholic encephalopathy. However, in yellow fever the condition is more severe and probably would be fatal even if no other lesions were present in the liver or other organs. Apparently the hemorrhages are of short duration, for there is usually no notable reaction on the part of the microglia or astrocytes, and few phagocytes are encountered. It is probable that these hemorrhages are not more than a day or two old.

Arsphenamine will occasionally cause a fatal hemorrhagic condition of the brain, with the brunt of the lesion being borne by the lenticular nucleus. Here, of course, there is damage to the blood vessels by the toxin, with resulting hemorrhage. Other poisons, such as cyanides, mercury, manganese, lead and phosphorus, may cause minute hemorrhages in the brain along with other changes. Carbon monoxide poisoning may cause hemorrhages in the brain, but in addition there are necrosis of the globus pallidus and thrombosis of small vessels. Snake venom and many other poisons, some used in industry, may cause small hemorrhages in the brain. We have just examined

Chart Showing Sites of Hemorrhage in the Brain in Twenty Cases of Yellow Fever

Case	Frontal Pole	Superior Frontal Gyrus	Paracentral Lobule	Temporal Lobe	Temporal Pole	Hippocampus	Occipital Lobe	Occipital Pole	Insula	Periventricular and Subthalamic Region at Level of Mamillary Body	Lenticular Nucleus	Midbrain	Pons	Medulla	Cerebellum	Spinal Cord	Olfactory Tract	Optic Nerve	Other Cranial Nerves
1	O	O	+	..	+	..	..	..	..	+	..	O	+	..	+	..	+	..	..
2	+	..	O	..	+	..	O	O	..	..	..	..	O	O	O	..	Badly decomposed		
3	..	+	..	..	..	+	+	..	+	+	+	+	+	..	+	..	..	..	..
4	+	+	+	+	..	..	..	+	O	+	O	..	+	+	+	..	O	..	..
5	+	O	+	..	+	..	..	O	..	O	..	O	O	O	O	..	..	..	..
6	+	O	+	+	+	O	O	..	O	+	..	O	..	+	O	..	..	..	..
7	..	O	+	..	+	..	..	+	O	+	O	O	O	O	+	..	..	..	..
8	..	+	+	+	..	O	..	+	O	+	..	+	..	+	+	..	+	+	..
9	..	..	O	..	+	+	+	..	..	+	..	+	..	..	+	..	..	..	..
10	..	..	O	+	..	O	O	..	..	+	O	O	..	O	O	..	O	..	..
11	O	O	..	..	+	..	..	O	O	+	O	..	..	..	..	+	..	..	..
12	..	O	..	+	..	+	..	..	..	+	O	O	..	O	O	..	..	O	O 3d
13	..	+	..	..	+	O	+	..	..	+	..	O	..	O	+	..	+	..	..
14	..	O	..	+	..	O	..	O	..	+	..	..	+	..	..	+	+	..	2d 5th 3d O 7th 4th 8th
15	..	..	O	..	+	..	O	..	..	..	..	+	..	+	O	..	..	+	..
16	..	O	..	..	+	O	..	+	..	..	O	..	..	..	+	O	O	..	..
17	O	O	..	+	+	O	..	..	..	O	+	O	..	O	+	..	O	O	..
18	..	+	..	..	..	+	..	..	..	+	+	+	..	+	+	..	+	+	3d O 5th
19	..	O	..	+	..	+	..	+	..	O	O	..	+	..	O	..	O	O	O 3d
20	O	..	..	..	+	..	..	O	..	+	..	O	O	O	..	..	O	..	..

the brain of a dog poisoned with metrazol, a convulsant drug used recently in the treatment of dementia praecox. We found perivascular hemorrhages. In cases of anoxemia of the brain small hemorrhages are common about the vessels. In cases of stasis of blood flow in the smaller vessels of the brain due to leukemia hemorrhage of the brain is not uncommon. In cases of acute anterior poliomyelitis small hemorrhages are usually encountered in the gray matter of the spinal cord as well as a perivascular exudation of lymphocytes and even of polymorphonuclear cells. In addition, however, there can be seen a specific effect of the virus on the nerve cells. In many cases of lethargic encephalitis large or small hemorrhages may be found in the lower parts of the brain. We have observed, also, perivascular hemorrhage in cases of pertussis.

In a true virus infection of the nervous system, lasting several days, there would undoubtedly be ample evidence of the infection in the brain in the form of lymphocytic accumulations in the Virchow-Robin spaces, in addition to other pathologic changes in the nerve cells, microglia, astrocytes and even the myelin sheaths at times. In these cases of yellow fever the hemorrhages were the most significant change discovered in the nervous system and were apparently a terminal event. They cannot be considered as evidence of neurotropism of the virus of yellow fever in man.

Most of these 20 cases of yellow fever occurred in the states of Paraná or Minas-Geraes in Brazil in January, February, March and April 1936. The average duration of illness was a little over six days, and the average age of the patients was 29 years. The youngest patient was about 5 years of age, and the oldest was 62.

The illness clinically was typical of yellow fever, beginning with headache and pains in the back. There were fever and much thirst, with epigastric pain, enlargement of the liver, some jaundice, often photophobia and congestion of the eyes and face. Black vomit and melena occurred in most instances, with albuminuria, oliguria and later anuria. Delirium and coma were present in some a day or so before death, and convulsions were present in a single case. Nervousness was noted in several.

The literature consulted threw little light on the question of the involvement of the nervous system in man. Jakob, Fialho and Villela [1a] reported on 14 cases in man in Rio de Janeiro, Brazil. No gross pathologic lesion was reported, but microscopically there was small cell infiltration of the leptomeninges, with macrophages containing blood pigment, also some edema of the pia. There was severe fatty degen-

1a. Jakob, A.; Fialho, A., and Villela, E. L.: Deutsche Ztschr. f. Nervenh. **3**:111, 1929.

eration of the nerve cells in the cortex and striatum. The authors noted proliferation of glia cells and some chromatolysis of nerve cells, with focal atrophies in the cortex and other tissues.

Stefanopoulo and Mollaret [2] reported observation of hemiplegia and optic neuritis in a case of yellow fever. The patient, however, recovered, so it is not known what the nature of the lesions in the brain was.

Findlay and Stern [3] noted ptosis of the left eyelid and partial facial paralysis in a patient who suffered from a severe but typical attack of yellow fever in Northern Nigeria.

Apparently in mice, monkeys and other animals true encephalitis may develop following inoculation with the virus of yellow fever under certain conditions. In the monkey (Rhesus) encephalitic symptoms are associated with microgial proliferation, perivascular infiltration and intranuclear inclusions (Findlay and Stern [3]). These authors concluded that there is evidence that neurotropic potentialities are inherent in the ordinary strain of the virus. Other authors have reported encephalitis in Macacus rhesus by inoculation of viscerotropic yellow fever (Goodpasture [4]; Penna [5]). Encephalomyelitis following vaccination against yellow fever has been reported by Lhermitte and Fribourg-Blanc [6] and by Dezest [7]). This, however, is quite another matter and is comparable to encephalitis following vaccination against smallpox. In the fatal case reported by Lhermitte and Fribourg-Blanc the patient died about fifteen months after vaccination. The lesions in this case resemble those seen in disseminated sclerosis.

SUMMARY

Preliminary studies were made on different regions of the brain in 14 cases of yellow fever in man. In 20 other cases of yellow fever in man the whole brain was available for study and in 3 of these cases the spinal cord as well. A detailed examination of the brains in these 20 cases is reported in this paper.

The chief lesion found in all of the brains studied was perivascular hemorrhage. These hemorrhages were most frequently found in the subthalamic and periventricular region at the level of the mamillary bodies. The temporal pole was next most involved and the cerebellum only slightly less so.

2. Stefanopoulo, G. J., and Mollaret, P.: Bull. et mém. Soc. méd. d. hôp. de Paris **50**:1463, 1934.
3. Findlay, G. M., and Stern, R. O.: J. Path. & Bact. **41**:431, 1935.
4. Goodpasture, E. W.: Am. J. Path. **8**:137, 1932.
5. Penna, H. A.: Am. J. Trop. Med. **16**:331, 1936.
6. Lhermitte, J., and Fribourg-Blanc: Rev. neurol. **65**:391, 1936.
7. Dezest, G.: Bull. Soc. path. exot. **30**:253, 1937.

Perivascular lymphocytic exudate was noted in 9 cases and with the exception of a single case this was slight.

Changes in the nerve cells were insignificant, and no inclusion bodies were seen.

Reactive changes in the microglia and astrocytes were slight.

From this study it is concluded that there is no definite evidence of neurotropism on the part of the virus of yellow fever in this series of cases.

PRIMARY SYSTEMIC AMYLOIDOSIS

INVOLVEMENT OF CARDIAC VALVES, JOINTS AND BONES, WITH PATHOLOGIC FRACTURE OF THE FEMUR

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Amyloidosis secondary to chronic suppurative disease or tuberculosis is relatively common. In contrast, the primary form of amyloid disease is distinctly rare. Yet within recent years primary systemic amyloidosis has become a recognized entity. A number of those who have reported cases of this disease have emphasized (1) the absence of known etiologic factors and (2) the tendency to involve smooth and skeletal muscle while organs such as the liver and spleen, usually affected in secondary amyloidosis, are uninvolved.

The following case of primary systemic amyloid disease is reported because of extensive involvement of the cardiac valves and of the joints and bones, with pathologic fracture of the femur.

REPORT OF A CASE

A 61 year old white woman was admitted to the Cleveland City Hospital Sept. 11, 1935, with complaints of fracture of the left hip and rheumatism. In 1924 she noticed for the first time swelling and stiffness of the fingers of the right hand. This was accompanied by tingling and burning sensations, which persisted for five years and then stopped. Eight years before admission she noted swelling and stiffness of the right wrist. After that all her joints gradually became stiff and swollen. This resulted in moderate disability, although there was no pain. During the past two years her tongue grew larger. About three months prior to admission she experienced sudden pain in the left hip while walking and fell down. She was put to bed and was unable to arise because of severe pain. Hospitalization was advised by a physician because of a fracture of the hip.

The past history revealed that the patient had been in good general health except for the disabilities mentioned. Her diet had always included a large amount of meat.

The temperature was 36.8 C. (97.9 F.); the pulse rate, 96; the respiratory rate, 21, and the blood pressure, 136 systolic and 88 diastolic. The patient was well nourished and not severely ill. The skin was smooth, glassy and free from wrinkles. The subcutaneous tissue and skeletal musculature throughout the body were firm. There was no icterus or superficial lymphadenopathy. The pupils

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reacted to light and accommodation. The fundi showed a slight degree of arteriosclerosis.

The tongue was enlarged and showed small nodules on the dorsum. On the inner aspects of the lips there were firm, elevated nontender nodules 1 cm. in diameter and of a grayish white appearance.

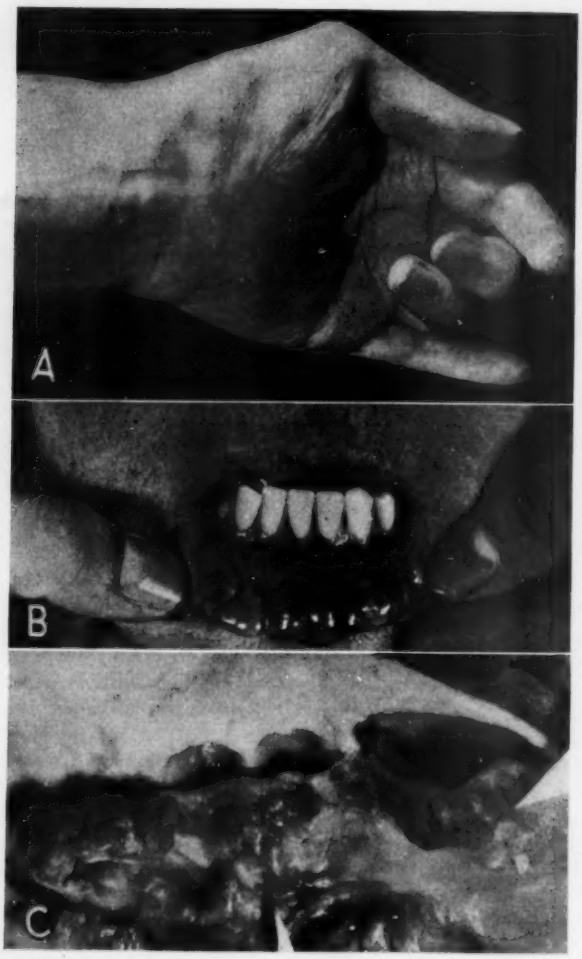

Fig. 1.—*A*, swelling and nodularity of the left wrist and palm; *B*, nodular lesions of the lower lip; *C*, amyloid involvement of the aortic valve.

The heart was not enlarged. The rate was normal and the rhythm regular. There was a loud blowing systolic murmur at the apex. The second sound at the base was harsh. The lungs and abdomen were normal. The liver and spleen were not palpable. Pelvic examination revealed firm nodular lesions from 1 to 2 cm. in diameter on the posterior vaginal wall just within the introitus.

All the joints were irregularly enlarged, firm to palpation and not tender. Much of the swelling and irregularity was apparently due to firm nodular masses involving the tendon sheaths and ligaments of the joints. Both limitation and rigidity of motion were present throughout. The shoulders seemed to be padded, and their bony markings were obscured. The wrists and elbows were irregularly swollen on the dorsal and volar aspects. In the antecubital spaces there were firm, elevated nodules 3 cm. in diameter, probably attached to the tendons and muscles. The left hip and thigh were diffusely enlarged. There was a firm immobile nontender mass extending down from the crest of the ilium and merging with the structures of the thigh.

The fingers of both hands were rigid and immobile except for a slight degree of passive flexion. The palms showed many subcutaneous nodular swellings. Both the thenar and the hypothenar eminences were obscured. On the dorsal aspect of the left hand there were nodules 1 cm. in diameter attached to the tendons.

There was no evident involvement of the vertebral column.

The urine was normal. An examination for Bence Jones protein was not made. The red blood cell count was 3,900,000; the hemoglobin content, 55 per cent; the white blood cell count, 6,200. The Wassermann reaction of the blood was negative. The serum phosphorus was 4.2 mg. and the serum calcium 9.4 mg. per hundred cubic centimeters. The phosphatase activity was 9.5 units. The serum cholesterol was 242 and 235 mg. per hundred cubic centimeters. Repeated examinations of the blood for urea nitrogen gave values within normal limits. Dextrose tolerance tests gave results as follows: during fasting, 98 mg. per hundred cubic centimeters of blood; at one half hour after administration of dextrose, 186 mg.; at one hour, 220 mg.; at two hours, 178 mg.; at three hours, 184 mg. The icterus index was 5. The blood uric acid was 4.1 and 4.3 mg. per hundred cubic centimeters; the total blood fat was 1.17 mg. per hundred cubic centimeters.

The congo red test showed on the first specimen 100 per cent, and on the second specimen 133 per cent, of the dye present (hemolysis).

The roentgenologic reports Sept. 16 to 17, 1935, were as follows: The left hip showed a fracture through the neck of the femur, with considerable fragmentation and absorption of the bone and slight upward displacement of the shaft. This fracture did not have the appearance of a recent one. Both hands, the left knee and the right shoulder showed generalized demineralization of all the bones. There appeared to be fragmentation about the greater tuberosity and a slight downward displacement of the head of the humerus. No other pathologic process was noted in these areas. The lumbar region of the spinal column showed similar demineralization of bone. Otherwise there was no significant change. There was no definite pathologic process in the right femur. A roentgenogram of the chest showed the cardiac shadow to be slightly increased in its transverse diameter, indicating cardiac hypertrophy and dilatation. The lungs were normal.

On admission the diagnosis of a pathologic fracture of the neck of the left femur was made. The underlying disease of the bone, as well as the nature of the involvement of the joints, was not clear.

The patient remained in the hospital for almost two and a half years before she died. For most of this period the clinical picture was puzzling. The joints, although swollen and deformed, were roentgenologically normal. No form of arthritis adequately explained the extensive involvement of the tendons. The diagnosis of gout was eliminated because of the lack of pain, the absence of tophi and the normal level of the uric acid in the blood. Although the patient was not diabetic, xanthoma tuberosum multiplex was considered because of the involvement of tendons, the subcutaneous and submucous nodules and the erosion of bone. However, the absence of cholesterol in the biopsy material excluded this possibility.

The first biopsy specimens consisted of a segment of extensor tendon from the right wrist and some small firm translucent masses from the joint space. Microscopically, the connective tissue of the tendon was largely replaced by homogeneous hyaline material. There was no inflammation, and no foam cells were

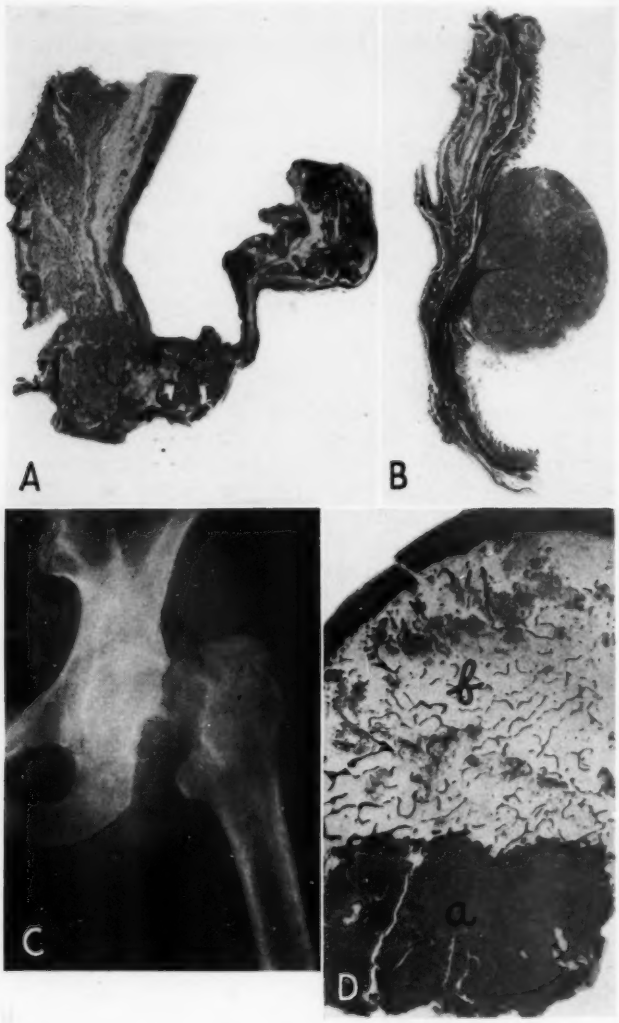

Fig. 2.—*A*, microscopic section through the midportion of the aortic cusp; × 3: amyloid involvement of pericardial wedge, ring and free portion of valve. *B*, amyloid nodules of the small intestine as shown in a microscopic section; × 3.87. *C*, pathologic fracture of the left femur. *D*, microscopic section of the head of the left femur showing (*a*) amyloid extending to the line of fracture and (*b*) a spongy portion of bone; × 2.32.

seen. With polarized light no anisotropic substance was found. The diagnosis was chronic productive and degenerative tenosynovitis. A second biopsy specimen taken from the right shoulder consisted of fragments of firm tissue of cartilaginous consistency, and the diagnosis was hyalinization of connective tissue. Later, tendon sheaths were removed from both wrists. Microscopic sections showed replacement of the tendon by a hyaline material which gave positive reactions for amyloid with methyl violet and congo red. There was no anisotropic substance. A diagnosis of chronic tenositis with amyloid change was made. Following the recognition of amyloid in the third biopsy specimen, the dermatologic consultants, who had been interested in the lesions of the tongue and lips, made a diagnosis of primary systemic amyloidosis.[1] Stains for amyloid in the earlier biopsy specimens were then found to be positive.

During the entire period of hospitalization the patient's condition remained stationary and, although largely bedridden, she was in fair health and had no additional complaints. With rest in bed, the pain in the left hip diminished as motion of the joint decreased and disappeared when ankylosis was complete. Subsequent roentgen examinations showed no appreciable change in appearance over that at admission. A subtrochanteric osteotomy was advised by the orthopedic consultant but was refused by the patient. She was given physical therapy and, with massage and gentle manipulation, gained some use of all her extremities. With a minimal amount of help, she was able to walk short distances on crutches and even resumed slight bearing of weight on the left leg.

A sudden attack of pain and blindness in the left eye occurred in December 1935, which was thought by the consultant to be due to embolism or spasm of the central artery. Following this episode there was permanent impairment of vision in the left eye.

In February 1938 the patient, after having been afebrile, began to have fever, cough and dulness to percussion over the entire right side of the chest. She died February 13 of confluent bronchopneumonia, after an illness of eight days.

The final clinical diagnosis was: primary systemic amyloidosis and terminal bronchopneumonia.

Autopsy.—A postmortem examination was made seven hours after death. There was no icterus or lymphadenopathy. The inner aspects of the lips and the tongue were as described already. The left lower extremity was 6 cm. shorter than the right. The skin throughout the body was smooth and glassy but on section showed no significant change in the epidermis or corium. The subcutaneous fat was firm and had a peculiar pale yellow color.

The skeletal muscles of the thoracic and abdominal walls were tense and rigid, and their fibers were extensively replaced by confluent nodular masses of translucent pearly gray lardaceous material. Gross tests of this material for amyloid by means of the iodine and sulfuric acid stain were strongly positive.

Externally all the joints were swollen and irregular in contour. The left elbow and left hip joints were selected for examination.

The entire articular capsule and synovial membrane of the left elbow joint were replaced by firm lardaceous grayish yellow tissue which extended into the joint space and filled the olecranon, coronoid and radial fossae. At the sites of attachment of the articular capsule to bone there was direct invasion by this tissue of the humerus, the olecranon and coronoid process of the ulna and the head of the radius.

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1. The patient was presented at a meeting of the Cleveland Dermatological Society, March 25, 1937 (Arch. Dermat. & Syph. **37**:330, 1938), as having primary systemic amyloidosis.

The left hip joint was partly encased in a huge solid mass of light yellow lardaceous tissue, which measured 10 by 5 by 4 cm. This tissue extended through the articular capsule to fill the joint space and had invaded the entire neck of the femur. There was a complete fracture through the middle of the neck, with separation of the fragments. The surface of the fracture showed numerous irregular tags of lardlike material. The articular surfaces of the joint were uninvolved except for the fovea capitis of the femur which was invaded by extension from the ligamentum teres. All the tendons adjacent to both the elbow and the hip joint were extensively replaced by firm translucent lardaceous tissue. The material in

Fig. 3.—Left femur. The upper arrow points to the line of fracture; the lower, to a mass of amyloid.

the joints, bones and tendons gave strongly positive reactions for amyloid with iodine and sulfuric acid.

The heart weighed 400 Gm. There were numerous firm raised pearly gray nodules, measuring 1 to 2 mm. in diameter, in the epicardium of the atria and in that of the base of the ventricles. The left atrial chamber was enlarged and had a thickened, rigid, leathery wall which did not collapse. Its muscle layer and endocardium were extensively replaced by firm grayish white tissue, and the entire endocardium presented a grayish yellow glistening nodular surface. The changes in the right atrium were similar but less extensive. The left ventricular chamber was small, while the right was considerably dilated. Both ventricles presented a slightly hypertrophic brownish red myocardium, which showed no fibrosis. There were occasional small grayish white deposits of amyloid, especially in a subendocardial position. The coronary arteries revealed no significant change.

All four cardiac valves showed nodular deposits of amyloid. The involvement of the mitral and aortic valves was particularly extensive. Numerous glistening pearly gray beadlike nodules were deposited in the base and free portions of the mitral leaflets, especially on their atrial surfaces near the free margins. As a result the leaflets were thickened, rigid and virtually immobile. Although there was no fusion of the commissures, the orifice of the valve was fixed in a markedly stenotic position. The chordae tendineae were slightly thickened but discrete. There were numerous nodular deposits in the free portions of the tricuspid leaflets. The aortic valve was severely deformed by the amyloid infiltration, and its cusps were thickened, retracted and immobile. Both the atrial and ventricular surfaces of the cusps, as well as the sinuses of Valsalva and the subvalvular endocardium, were studded with discrete and confluent hard pearly white nodular excrescences. Although the commissures were not fused, the valvular orifice was definitely stenotic. The pulmonic valve was the least affected, showing only a few nodular deposits on the ventricular aspects of the cusps.

The aorta and pulmonary arteries were the seat of moderate arteriosclerotic change but were otherwise not unusual.

The lungs showed confluent bronchopneumonia, which was especially severe in the lower lobes. The crepitant portions were grayish brown and were firm and indurated to palpation. Moderate arteriosclerotic change of the small pulmonary arteries was present. The bronchi presented a hyperemic brownish red granular mucosa but their lumens contained no exudate. The hilar lymph nodes were small and showed nothing unusual on section.

The liver and spleen weighed 1,250 Gm. and 150 Gm., respectively, and were the seat of passive hyperemia. Otherwise they showed nothing unusual.

No significant changes were noted in the gallbladder and biliary ducts, pancreas, adrenals, urinary tract, pelvic organs, breasts, thyroid and parathyroids, diaphragm, esophagus, stomach, duodenum and brain. There was no abdominal lymphadenopathy.

The posterior wall of the vagina just within the introitus showed several small elevated nodular masses of firm grayish white tissue from 1 to 2 cm. in diameter.

The midportion of the jejunum and the large intestine from the ascending colon to the sigmoid showed in their walls numerous discrete pearly gray nodules 0.5 to 5 mm. in diameter. These lesions cut with resistance. Grossly they appeared to be confined to the muscle layer and were covered by intact mucosal and serosal surfaces. Gross tests for amyloid were positive.

The ribs and vertebrae were intact and presented the usual red marrow. The marrow of the upper half of the left femur was hyperplastic, friable and pale red.

Microscopic Examination.—The histologic preparations were stained by the hematoxylin and eosin, Van Gieson and azocarmine methods. Both fresh tissue and tissue fixed in solution of formaldehyde were examined for amyloid with congo red, methyl green and iodine–sulfuric acid stains.

Both ventricles of the heart revealed hypertrophy of their fibers. The small blood vessels, especially the arteries, showed subendothelial and medial deposition of an eosin-staining homogeneous material, which was positive for amyloid. The muscle was occasionally interrupted by nodular deposits of the same substance. The involvement of the left atrium was extensive. In one area of deposit in the endocardium there was progressive transformation of amyloid through the stage of cartilage to bone. The bony trabeculae enclosed a fatty marrow, which showed a few hemopoietic cells.

Analysis of Twenty-Two Cases of Primary Systemic Amyloidosis as Recorded in the Literature

Authors	Year	Age	Sex	Symptoms	Clinical Diagnosis	Total Duration of Disease	Cause of Death	Distribution of Amyloid at Autopsy
Wild [8a]	1886	56	♀	History incomplete	Pulmonary emphysema, cardiac insufficiency	Not known	Erysipelas	Tongue, heart, heart valves, gastrointestinal tract, lymph nodes, bladder, lungs, pericardium, peritoneum
Steinhaus [8b]	1902	40	♂	Intestinal hemorrhages; dyspnea; vomiting; obstipation	Carcinoma of pylorus	6 mo.	Shock	Heart, stomach, pylorus, intestine
Ritter [8c]	1908	50	♂	Pain in sacral region and extremities; swelling of tongue, dysphonia, and dysphagia; constipation	Carcinoma of tongue, inanition	2 yr.	Bronchopneumonia, cardiac insufficiency	Tongue, stomach, intestine, ? heart
Beneke [8d]	1922			Data not available				Heart, tendons, ligaments and capsules of joints, large veins, pulmonary arteries and veins
Königstein [5]	1925	60	♂	Pain in shoulders, arms and hands; weakness; swelling of tongue, dysphonia and dysphagia; constipation and diarrhea	Generalized amyloidosis	2 yr.	Ileus, pneumonia	Tongue, heart, skin, skeletal muscles, gastrointestinal tract, adipose tissue, bladder, urethra, corpus cavernosum of penis, superior vena cava
Lubarsch [3a] (3 cases)..	1927	53	♂	Headaches and dizziness; lameness	Myotonia, scleroderma, carcinoma of tongue	8 yr.	Pneumonia, empyema	Tongue, heart, skin, skeletal muscles, gastrointestinal tract, lymph nodes, bladder, prostate, seminal vesicles, testes, epididymides, pharynx, esophagus, lungs, pleurae, diaphragm, peritoneum, dura, adrenals, capsule and tendons of knee and hip joints
	1927	66	♀	Purpura of skin; headaches; dyspnea	Thrombopenic purpura, cystopyelitis, bronchitis	14 mo.	Cardiac insufficiency	Tongue, heart, skin, gastrointestinal tract, esophagus, lungs
	1927	45	♂	Hematemesis; anorexia; constipation, weakness and pain after meals	Carcinoma of stomach	1 yr.	Death followed laparotomy	Heart, gastrointestinal tract, lungs, lymph nodes (thoracic, abdominal and inguinal), mesentery, prostate, seminal vesicles, testes, epididymides, spleen (pyloric ulcers)
Picchini and Fabris [8g]	1930	51	♀	Swelling of tongue, dysphonia and dysphagia; dyspnea; swelling of external genitalia	Scleromegaly with multiple hemorrhages and lymphadenopathy	3 yr.	Death followed tracheotomy to relieve dyspnea	Tongue, heart, lips, palate, larynx, trachea, muscles of neck
Warren [8h]	1930	54	♀	Canker sores of tongue; swelling of tongue and cheeks	Leukoplakia of tongue	20 mo.	Bronchopneumonia	Tongue, heart, skeletal muscles, gastrointestinal tract, diaphragm, cheeks, esophagus, gallbladder, urinary bladder, uterus, arteries and veins
Pick [81]	1931	54	♂	Disturbances of motion; swelling of tongue and dysphagia	Myotonia, scleroderma, carcinoma of tongue	Not known		Tongue, heart, gastrointestinal tract, lungs, esophagus, pharynx, serosal surfaces

Gottron [8j]	1932	46	♀	Difficulty in standing, walking; swelling of tongue, dysphagia and dysphonia; constipation	Systemic amyloid disease	3 yr.	.....	Tongue, skin, skeletal muscles, gastrointestinal tract (clinical evidence)
Gerstel [8k]	1932	52	♀	Swelling of tongue and dysphagia; dyspnea; diarrhea—melena; constipation; weakness	Carcinoma of floor of mouth, intestinal paralysis	2 yr.	Cardiac failure	Tongue, heart (subepicardium), gastrointestinal tract, skeletal muscles (neck and gluteal region), mouth, soft palate, pharynx, esophagus, periesophageal fibroadipose tissue, skin of neck, mesentery, diaphragm, adventitia of large vessels
Mollow and Lebell [8l]	1932	60	♂	Abdominal pain, meteorism, constipation; swelling of tongue, dysphonia and dysphagia; difficulty in walking	Paralysis agitans, hypertonia of muscles, stenosis of sigmoid colon	16 mo.	Cardiac failure	Tongue, heart, gastrointestinal tract, skeletal muscles, skin, diaphragm
Von Bonsdorff [8m]	1933	51	♂	Stiffness of tongue; large tumors about joints; weakness	Local amyloid disease	1½ yr.	Bronchopneumonia	Tongue, mouth, tendons, joints, bones, lymph nodes (axillary)
Strauss [8n]	1933	72	♂	Tendency to stoop; dyspnea; cervical adenopathy	Carcinoma of lung, carcinomatosis	1 yr.	Not stated	Heart (epicardium), pericardium, lungs, lymph nodes (universal), connective tissue of mediastinum, appendix, periaortic fibroadipose tissue
Michelson and Lynch[8o]	1934	54	♂	Pain in lumbar region and joints, limitation of motion; swelling of tongue, dysphonia; constipation	Systematized amyloidosis	18 mo.	Intestinal hemorrhage, ileus	Skin, tongue, buccal and anal mucosa (clinical evidence)
Gaupp [4]	1934	53	♀	Weakness, fatigue, difficulty in walking	Not given	2 yr.	Patient died suddenly	Tongue, heart, skin, cervical lymph nodes, aorta, pulmonary arteries, skeletal muscles, uterus, kidneys
Perla and Gross [2a]	1935	53	♀	Dyspnea, cough, pain in chest, loss of weight, weakness	?Carcinoma of lung, congestive heart failure	Several months	Sudden circulatory collapse	Tongue, heart, gastrointestinal tract, lungs, diaphragm, uterus, kidneys
Reimann and others [8r]	1935	41	♀	Pain in extremities; swelling of tongue and submental region, dysphagia, dysphonia, dyspnea; amenorrhea	Amyloid disease	2 yr.	Peritonitis	Tongue, heart, lungs, esophagus, pelvic organs (clitoris, vulva, vagina, uterus), mediastinum
Weber and others [8s]	1937	48	♀	Pain in fingers and shoulders; swelling of tongue and neck, dysphonia; fatigue; intermittent claudication	Systematized atypical amyloidosis	2 yr. 4 mo.	Patient discharged from hospital after resection of tongue	No autopsy
DeNavasquez and Treble [3b]	1938	36	♂	Diarrhea; muscular weakness; dyspnea; dizziness on exertion	Beriberi, chronic enteritis	2 yr. 2 mo.	Streptococcic pharyngitis, cardiac failure	Heart, intestines, posterior root and sympathetic ganglions, peripheral nerves, thyroid, adrenals, testes, spleen, arterioles (generalized)
Haenisch [8t]	1938	58	♀	.....	Carcinoma of bladder, papillomatosis of bladder; myocardial disease, bronchopneumonia	.....	Cardiac failure	Heart, tongue, urinary bladder
Koletsky and Stecher	1938	61	♀	Swelling of joints, limitation of motion; fracture of left hip; swelling of tongue	Primary systemic amyloidosis	14 yr.	Bronchopneumonia	Tongue, heart, heart valves, skeletal muscles, intestines, joints, bones, tendons, vagina, lips

The pericardial wedge and ring of the aortic valve showed numerous nodular hyaline masses. All the layers of the free portion, especially the fibrosa, were likewise involved, and the tip of the valve showed confluent deposits of this substance. The elastic fibers were essentially intact, although frequently compressed and distorted by the amyloid. There was no inflammatory reaction, and only vessels of the sinusoidal type free from amyloid disease were present. The mitral ring and valve showed similar extensive nodular deposits of amyloid. There was no vascularity or inflammation.

The lungs showed areas of edema and bronchopneumonia. The alveolar walls were thick, owing to an increase in fibrous tissue, and numerous "heart failure" cells were present within the alveolar spaces. The walls of the small arteries were distinctly thickened. Occasionally a small artery or vein showed subendothelial deposition of amyloid.

In the liver and spleen severe passive hyperemia was observed. No amyloid involvement was found.

The vagina showed nodular hyaline masses in the tunica propria of the mucosa and in the underlying muscle. The small arteries in the neighborhood of these deposits showed amyloid infiltration. There was a slight amount of hemorrhage.

In the intestines the longitudinal layer of muscle was interrupted by nodular masses of homogeneous acidophilic material. The circular layer was compressed but otherwise uninvolved. There was irregular replacement of the muscularis mucosae by amyloid, which extended into the tunica propria. The small arteries, particularly those of the submucosa, were the seat of severe amyloid deposition. In some sections the blood vessels showed extensive infiltration while the muscle was free from disease.

The epidermis and corium were normal. Nodular deposits of amyloid were present deep in the subcutaneous tissue adjacent to the underlying fascia and muscle. Several nodules showed areas of transformation of amyloid into cartilage.

Deposits of acidophilic homogeneous material were found in the connective tissue of the tunica propria and underlying muscle of the lip.

The tumor mass of the left hip was composed of confluent lobulated masses of amyloid. Some of the lobules revealed blue-staining areas which suggested early cartilage formation. The vessels consisted of endothelial slits surrounded by amyloid.

The fibers of the skeletal muscle were spread apart and in some areas replaced by diffuse nodular deposits of amyloid. Most of the muscle cells appeared normal even when surrounded and compressed by this substance. Some were atrophic as a result of pressure. In several areas bands of amyloid in the interstitial connective tissue seemed to invaginate and possibly invade the fibers. The small arteries in the interstitial stroma showed extensive amyloid infiltration of their walls.

At the line of fracture of the neck of the left femur the bone was completely replaced by amyloid. The boundary between amyloid and the uninvolved cancellous bone of the head of the femur was sharp. The latter showed fatty marrow with islands of hemopoiesis. Within the amyloid tissue were several small foci of bone formation similar to that in the left atrium. Marrow from the upper portion of the left femur showed myeloid hyperplasia and no amyloid. Histologic section of a vertebra also showed myeloid hyperplasia. Some of the small arteries were the seat of severe amyloid deposition, but their lumens were not reduced. The reticulum and bony trabeculae were not unusual.

As regards the ligaments and tendons, the connective tissue bands were disrupted and replaced by nodular masses of acellular acidophilic material. Within the nodules were sinusoid-like vessels showing widely patent lumens filled with red blood cells and intact endothelial linings. In the uninvolved portions of connective tissue the vessels showed no change.

In the left eye the central artery of the retina showed an extensive deposit of amyloid, but its lumen was amply patent. There was no change in the retina. On the surface of the sclera the posterior ciliary vessels also showed severe amyloid involvement with virtual occlusion of the lumens.

Histologic sections of tracheobronchial lymph node, gallbladder, aorta, kidney, breast, uterus, pituitary, brain and meninges presented no significant change and no amyloid disease of the vessels.

Microscopic study of the pancreas, adrenal, esophagus, mesoappendix, thyroid and parathyroids showed amyloid infiltration of an occasional small artery. Otherwise there was nothing unusual.

The material in the various areas of deposit gave consistently positive reactions for amyloid. In the smaller nodules the stains were homogeneous, but the larger masses in the region of the joints showed coarse clumps of amyloid surrounded by lighter staining, almost nonspecific hyaline material. Some nodules presented a whorl-like appearance, as if the material had been deposited in irregularly concentric layers. Foreign body giant cell reaction at the periphery was frequent. The vessels within the nodules consisted of endothelial vascular slits completely surrounded by amyloid. There was no cellular infiltration. Special stains showed only a slight deposit of fat in the form of small droplets.

Amyloid involvement of blood vessels occurred chiefly in the small arteries. Usually the entire wall of the vessel was transformed to a homogeneous hyaline tissue without significant reduction of the lumen.

The final pathologic diagnosis was: primary systemic amyloidosis, with involvement of the skeletal muscles, lips, tongue, tendons, joints, bones, heart, and valves, intestinal tract and vagina; pathologic fracture of the left femur; cardiac hypertrophy and dilatation; chronic passive hyperemia of the lungs, liver and spleen; pulmonary arteriosclerosis, and bronchopneumonia.

COMMENT

Amyloid disease may be subdivided into four groups as follows: (1) secondary amyloidosis, (2) primary amyloidosis, (3) amyloidosis associated with multiple myeloma and (4) tumor-forming amyloidosis. Of these, sceondary amyloidosis is relatively common and usually follows long-standing disease, such as tuberculosis or chronic suppuration. The liver, spleen, kidneys and adrenals are particularly involved, and the amyloid is deposited in a subendothelial position in the walls of capillaries and arterioles. In this form the amyloid gives typical staining reactions. Primary amyloidosis is characterized by absence of known etiologic factors. The amyloid is found in mesodermal tissue, smooth and skeletal muscle, the cardiovascular system and the gastrointestinal tract, while the organs usually affected in secondary amyloidosis, such as the liver and spleen, are uninvolved. The staining reactions of the amyloid are variable, and the substance tends to be deposited in nodular

form (Lubarsch). Amyloid disease associated with, and presumably secondary to, multiple myeloma has a distribution and character similar to the primary form. Tumor-forming amyloid is usually of the primary type or associated with multiple myeloma. It is characterized by the formation of small or large, solitary or multiple tumors, which occur especially in the larynx but also in various other sites such as the tongue, pharynx, eye, bladder and bone.

This classification of amyloidosis is satisfactory, since the differences between the various types are in general constant. However, overlapping of characteristics and atypical distribution may occur. For example, amyloid disease of unknown causation (i. e., primary) may involve chiefly the liver, spleen, kidneys and adrenals.[2] In 3 cases of primary systemic amyloidosis the amyloid was not confined to mesodermal structures but involved parenchymatous organs: the spleen [3] and kidneys.[4] Amyloidosis secondary to chronic disease may show infiltration of mesodermal tissues. In a case of Königstein's [5] amyloid disease attributed to chronic nephritis involved the tongue, heart, skin, spleen, lungs and nasal mucosa. Feller [6] reported the case of a 40 year old woman with urinary calculi who died in uremia and at autopsy showed amyloid of the tongue and joints. In this instance the amyloid was presumably secondary to a severe chronic infection of the urinary tract. A similar case is that of Lengh,[7] whose patient was a 46 year old woman with chronic pyelonephritis, renal calculi and amyloid disease of the tongue and joints. Death in this case was also due to uremia.

Primary amyloidosis may be divided into localized and systemic forms. In the localized variety the amyloid is limited to one or possibly two organs, or there is extensive involvement of one organ with slight and relatively insignificant deposits in other organs. The systemic variety is characterized by a generalized distribution of amyloid in mesoblastic structures of the body, especially in smooth and skeletal muscle, and the resulting picture is that of a systemic disease of the mesoderm.

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2. (*a*) Perla, D., and Gross, H.: Am. J. Path. **11**:93, 1935. (*b*) Bannick, E. G.; Berkman, J. M., and Beaver, D. C.: Arch. Int. Med. **51**:978, 1933. (*c*) Gerber, I. E.: Arch. Path. **17**:620, 1934. (*d*) Husten, K.: Virchows Arch. f. path. Anat. **248**:450, 1924. (*e*) Edens, E.: ibid. **184**:137, 1906.

3. (*a*) Lubarsch, O.: Virchows Arch. f. path. Anat. **271**:867, 1929. (*b*) DeNavasquez, S., and Treble, H. A.: Brain **61**:116, 1938.

4. Gaupp, A.: Ein Fall von generalisierter atypischer Amyloidose (Paramyloidose), Med. Dissert., Munich, 1934.

5. Königstein, H.: Arch. f. Dermat. u. Syph. **148**:330, 1925.

6. Feller: Centralbl. f. allg. Path. u. path. Anat. **63**:123, 1935.

7. Lengh, F.: Zentralbl. f. allg. Path u. Anat. **69**:1, 1937.

A survey of the literature to date revealed 23 cases of primary systemic amyloidosis.[8] These, together with our case, are analyzed in the accompanying table. Wild [8a] described the first case in 1886. The patient was a 56 year old woman with pulmonary emphysema and cardiac insufficiency, who died of erysipelas. At autopsy amyloid was found in the tongue, heart, gastrointestinal tract, lymph nodes, bladder, lungs, pericardium and peritoneum. The clinical data in this case are incomplete, but the author stresses the fact that none of the usual preceding etiologic factors, such as chronic infection or tuberculosis, could be demonstrated. Following Wild's case there were reported only 4 sporadic cases within the next forty-one years, those reported by Steinhaus,[8b] Ritter,[8c] Beneke [8d] and Königstein.[5] Beneke [8d] described 6 cases of primary local amyloid disease of the heart. One of the patients, however, showed in addition diffuse involvement of tendons and of the ligaments and capsules of the joints, so that the condition is probably best classified as systemic amyloid disease. Königstein [5] was the first to report lesions of the skin, and in his case the diagnosis of generalized amyloidosis was established clinically by means of a biopsy on skin. In 1929 Lubarsch [3a] described 3 cases of "atypical" or systematized amyloidosis and clearly separated this form of amyloid disease from the typical and more common secondary type. From 1929 to the present time, namely within approximately the last decade, 15 cases have been reported in the literature.[9]

Data as to the distribution with reference to age and sex are given in the table. In age the patients ranged from 36 to 72 years, with an average age of 52 years. There were 11 males and 11 females. As far

8. In chronologic order, these cases were reported by the following authors: (*a*) Wild, C.: Beitr. z. path. Anat. u. z. allg. Path. **1**:177, 1886. (*b*) Steinhaus, F.: Ztschr. f. klin. Med. **45**:375, 1902. (*c*) Ritter, E.: Virchows Arch. f. Path. Anat. **192**:536, 1908. (*d*) Beneke, R.: Centralbl. f. allg. Path. u. path. Anat. **33**:240, 1922. (*e*) Königstein.[5] (*f*) Lubarsch.[3a] (*g*) Picchini, L., and Fabris, A.: Arch. per le sc. med. **54**:551, 1930. (*h*) Warren, S.: Am. J. Path. **6**: 161, 1930. (*i*) Pick, L.: Klin. Wchnschr. **10**:1515, 1931. (*j*) Gottron, H.: Arch. f. Dermat. u. Syph. **166**:584, 1932. (*k*) Gerstel, G.: Virchows Arch. f. path. Anat. **283**:466, 1932. (*l*) Mollow, W., and Lebell: Wien. Arch. f. inn. Med. **22**:205, 1932. (*m*) von Bonsdorff, B.: Finska läk.-sällsk. handl. **75**:447, 1933. (*n*) Strauss, A.: Virchows Arch. f. path. Anat. **291**:219, 1933. (*o*) Michelson, H. E., and Lynch, F. W.: Arch. Dermat. & Syph. **29**:805, 1934. (*p*) Gaupp.[4] (*q*) Perla and Gross.[2a] (*r*) Reimann, H. A.; Koucky, R. F., and Eklund, C. M.: Am. J. Path. **11**:977, 1935. (*s*) Weber, F. P.; Cade, S.; Stott, A. W., and Pulvertaft, R. J. V.: Quart. J. Med. **6**:181, 1937. (*t*) Haenisch, R.: Frankfurt. Ztschr. f. Path. **52**:107, 1938. (*u*) DeNavasquez and Treble.[3b]

9. Picchini and Fabris.[8g] Warren.[8h] Pick.[8i] Gottron.[8j] Gerstel.[8k] Mollow and Lebell.[8l] von Bonsdorff.[8m] Strauss.[8n] Michelson and Lynch.[8o] Gaupp.[4] Perla and Gross.[2a] Reimann and others.[8r] Weber and others.[8s] DeNavasquez and Treble.[3b] Haenisch.[8t]

as is known all of the patients were white. The duration of the disease from the onset of symptoms to death averaged approximately two and one-half years. In Lubarsch's first case the duration was eight years, and in our case it was fourteen years, which is the longest on record. In the majority of the patients the condition grew progressively worse, with a fatal termination due to intercurrent infection.

The clinical manifestations of primary systemic amyloidosis are fairly uniform. One of the most constant findings is enlargement of the tongue due to amyloid infiltration. While pain may be absent, the swelling and immobility of the organ result in dysphonia and dysphagia. In a number of cases the macroglossia has been mistaken for carcinoma. In extreme instances the patient is unable to close the mouth.[10] The enlargement of the tongue is occasionally accompanied by swelling of the neck and face due to amyloid involvement of the skin, subcutaneous tissue or muscles. This may produce a fixed staring expression resembling that of a patient with paralysis agitans.[8l] A common symptom is progressive weakness and fatigue due to involvement of skeletal muscles. The clinical picture then simulates myotonia, although the muscles are enlarged and firm. In the case reported by DeNavasquez and Treble,[3b] however, muscular weakness was caused by amyloid infiltration of the nerves. Disturbances in gait and limitation of motion are frequent and due to involvement of muscles, tendons and joints. In 5 cases there were complaints of pain in the back, extremities or region of the joints. Pain in the finger tips [11] and hardening of the finger pads [8a] may occur.

The skin was involved in 8 of the 24 cases. Königstein [5] described an extensive eruption of sharply defined firm opalescent papules about the eyes and mouth and on the neck, trunk, extensor aspects of the extremities and fingers. Similar lesions of nodular type were also noted by Mollow and Lebell.[8l] In the patient observed by Michelson and Lynch [8o] an eruption of the eyelids developed in the form of firm translucent waxy papules. In 2 cases reported by Lubarsch,[8a] as well as in those reported by Gerstel [8k] and Gaupp,[4] the cutaneous lesions were of sclerodermic type. Gottron [8j] described papular lesions of the skin of the thorax and abdomen in addition to a plaquelike scleroderma.

Infiltration of the intestine leads most frequently to constipation (8 cases). Other symptoms include diarrhea, abdominal pain, vomiting and meteorism. Intestinal hemorrhage occurred in 3 cases,[12] in 2 of which it was either the immediate cause of death or a contributing factor. In Steinhaus' [8b] case, in which the condition simulated carcinoma of the

10. Gottron.[8j] Gerstel.[8k]

11. Ritter.[8e] Mollow and Lebell.[8l]

12. Steinhaus.[8b] Gerstel.[8k] Michelson and Lynch.[8o]

pylorus, the bleeding was attributed to amyloid disease of the vessels. In the case reported by Michelson and Lynch [8o] death was due to intestinal hemorrhage and ileus. The third case described by Lubarsch [3a] concerned a 45 year old man whose chief complaint was hematemesis. There was a one year history of pain following meals, with anorexia and constipation. At autopsy there was amyloid disease of the stomach with a number of small pyloric ulcers showing a moderate amount of amyloid. Gottron [8j] obtained roentgen evidence of amyloid involvement of the stomach in the form of thickening of the mucosa and diminished peristalsis. Gerstel's [8k] patient had severe diarrhea with melena and then intestinal obstruction. In the case observed by Mollow and Lebell [8l] there were persistent abdominal pain and constipation due to partial obstruction of the sigmoid colon.

Amyloid involvement of the heart occasionally leads to cardiac insufficiency. However, symptoms referable to cardiac failure may be difficult to evaluate. For example, dyspnea may be caused by involvement of the trachea, lungs or mediastinum or by the secondary anemia which is occasionally present. In the reports of 2 cases, however, the authors [13] stated definitely that the extensive replacement of the cardiac muscle by amyloid contributed to the clinical picture of failure. Although the small arteries are often diffusely involved, the blood pressure is either normal or slightly low.[8n] Intermittent claudication was present in the case observed by Weber and his colleagues.[8s]

Purpura is a frequent symptom and is presumably due to amyloid infiltration of blood vessels. The hemorrhages are most common in the skin but also occur in the tongue and mucous membranes. There may be a tendency to bruise readily.[14] Hematemesis [3a] and melena [12] have been described, and in the case of Picchini and Fabris [8g] there were hemorrhages in the eyegrounds. Hematuria was present in 2 cases.[15] Extensive cutaneous hemorrhages occurred in Lubarsch's [3a] second case, in which a diagnosis of thrombocytopenic purpura was made. However, since the platelet count was 189,000 and the bleeding time three and one-half minutes, this diagnosis appears doubtful, and the bleeding may have been secondary to amyloid disease.

Other symptoms and signs include amenorrhea due to amyloid infiltration of the uterus [8r] and dyspnea caused by extensive laryngeal involvement with obstruction.[8g] Difficulty in micturition, impotence and pupillary changes in the patient observed by DeNavasquez and Treble [3b] were ascribed to infiltration of the autonomic nervous system.

The distribution of the amyloid at autopsy was often very extensive. The most frequently affected organs were the tongue, heart, stomach,

13. Perla and Gross.[2a] DeNavasquez and Treble.[8b]
14. Michelson and Lynch.[8o] DeNavasquez and Treble.[3b]
15. Picchini and Fabris.[8g] DeNavasquez and Treble.[8b]

intestine and skeletal muscles. In 17 cases there was involvement of component organs of the cardiorespiratory, gastrointestinal and skeletal systems. The tongue was the seat of amyloid deposits in 20 of the 24 cases. Involvement of the heart occurred in 19 cases but in 2 of these was confined to the subepicardium.[16] The weights of the affected hearts were usually within normal limits or slightly increased. However, the hearts of 2 patients weighed 545 and 750 Gm. respectively.[17] Infiltration of the valves of the heart occurred in the instance reported by Wild,[8a] as well as in the present case. There was amyloid in the gastrointestinal tract in 16 cases and in the skin in 8 cases. Involvement of the lungs, diaphragm, lymph nodes and serosal surfaces such as the peritoneum, pericardium and pleura was not uncommon. The involvement of lymph nodes may be universal [18] or localized.[19] Infiltration of skeletal muscles occurred in 10 cases, of tendons and joints in 4 and of bone in 2. Other sites of deposit include the lips, interior of the mouth, pharynx, esophagus, larynx, trachea, bladder, anus and practically the entire genital tract of the male and of the female. Involvement of the dura mater [3] and of the vessels of the choroid plexus [20] and pia mater [8l] has been reported, but there is no instance of deposit in the brain. DeNavasquez and Tréble [3b] described infiltration of the posterior roots and sympathetic ganglions from the cervical, thoracic and lumbar regions and of peripheral nerves such as the ulnar, sciatic and peroneal. In the ganglions amyloid was found in nodular form between the ganglion cells and axis-cylinders. In many of the cases there was widespread involvement of small blood vessels, especially arteries, which in some instances included vessels in the liver, spleen and kidneys.

Primary systemic amyloidosis is a rare disease and consequently difficult to diagnose clinically unless its characteristics, distribution and fairly uniform group of symptoms and signs are kept in mind. It may simulate carcinoma of the tongue, scleroderma, myotonia, arthritis or any combination of these. In the case described by DeNavasquez and Treble [3b] the association of cardiac failure, peripheral neuritis and diarrhea suggested beriberi. Involvement of the lungs may be mistaken for carcinoma.[21] In 6 of the 23 cases collected the diagnosis was established by means of biopsy of tissues from various sites including the skin,[22] tongue [23] buccal mucosa,[8o] skeletal muscle [24] vagina [8r] and finger tip.[8s]

16. Gerstel.[8k] Strauss.[8n]
17. Strauss.[8n] DeNavasquez and Treble.[3b]
18. Lubarsch.[3a] Strauss.[8n]
19. von Bonsdorff.[8m] Gaupp.[4]
20. Mollow and Lebell.[8l] DeNavasquez and Treble.[3b]
21. Strauss.[8n] Perla and Gross.[2a]
22. Königstein.[5] Gottron.[8j] Reimann and others.[8r]
23. von Bonsdorff.[8m] Michelson and Lynch.[8o] Reimann and others.[8r] Weber and others.[8s]
24. Gottron.[8j] Weber and others.[8s]

With respect to biopsies it should be remembered that the specific stains for amyloid may be atypical in color or faint in intensity; they may fade rapidly; in some instances they are entirely negative. In several cases the amyloid gave positive reactions with methyl violet but was weak or negative to iodine and sulfuric acid and to congo red.[25] In Gottron's [8j] case, however, the congo red stains were positive and the methyl violet negative. The reactions may be typical in some areas of deposit and negative in other areas.[26] In some of the cases, including our own, the staining reactions were entirely typical.[27] Aid in diagnosis may be obtained by the use of the intravenous congo red test. Both positive [28] and negative [29] results have been reported with this method in cases of primary systemic amyloidosis. In respect to diagnosis, Lipstein's [30] recent evaluation of the congo red test for amyloidosis is of interest. In a series of 125 tuberculous patients he correlated the percentage of dye absorbed clinically with the presence or absence of amyloid at autopsy and concluded that the tests could be interpreted as confirmatory evidence of amyloid disesae only when the percentage of dye absorbed was 90 or higher.

That multiple myeloma is frequently accompanied by amyloid disease is well known. This association is obviously too frequent to be merely coincidental. Magnus-Levy [31] expressed the belief that the Bence Jones protein in cases of myeloma is chemically related to amyloid. Since the amyloid in cases of myeloma has the distribution and character of that in the primary form of the disease, the possibility of myeloma must be considered both clinically and at autopsy in every instance of amyloid disease of unknown cause. This is particularly true of amyloid involvement of joints and bone. In Glaus's [32] case apparently primary systemic amyloid disease involving the tongue, heart, gastrointestinal tract, skin, skeletal muscles, lungs and bone marrow was shown at autopsy to be associated with multiple myeloma. Among the cases of primary systemic amyloidosis tabulated there is presumably no instance of an association of amyloidosis with myeloma. A number of authors have emphasized that they found no evidence of such a tumor. Myeloma was ruled out in our case. In the patient observed by Michelson and Lynch [8o] the roentgenograms showed demineralization of bone suggesting disuse atrophy and were not diagnostic of myeloma. However, because of

25. Mollow and Lebell.[8l] Reimann and others.[8r] Weber and others.[8s]
26. Lubarsch.[3a] Steinhaus.[8b]
27. Königstein.[5] Warren.[8h] Gaupp.[4] DeNavasquez and Treble.[3b]
28. Gottron.[8j] Michelson and Lynch.[8o]
29. von Bonsdorff.[8m] Reimann and others.[8r]
30. Lipstein, S.: Am. J. M. Sc. **195**:205, 1938.
31. Magnus-Levy, A.: Ztschr. f. klin. Med. **126**:62, 1934.
32. Glaus, A.: Virchows Arch. f. path. Anat. **223**:301, 1917.

Bence Jones proteinuria, the possibility of myeloma cannot be definitely excluded in their case without autopsy. As far as is known, Bence Jones proteinuria was not present in any other case, although examinations for this substance have apparently been performed in only a few instances.[33]

In the present case of primary systemic amyloidosis there was widespread involvement of joints and secondarily of bone. The patient came under medical observation because of a pathologic fracture. The fourteen year duration of the disease is noteworthy. During the terminal two and one-half year period of clinical observation the patient remained in fair general health. In the following respects the condition in the present case is fairly typical of primary systemic amyloid disease: 1. There was no preceding or concurrent disease. 2. The involvement was limited to mesodermal structures, such as smooth and skeletal muscle and joints. 3. The amyloid was deposited in nodular form as illustrated in the intestinal and cardiac lesions. The amyloid gave consistently positive staining reactions with the congo red, methyl green, and iodine and sulfuric acid methods. Moreover, the results were positive with fresh tissue, with tissue fixed in solution of formaldehyde and with paraffin-embedded tissue. Special stains showed an insignificant amount of fat in the amyloid. This has been the general experience in other cases, although the presence of considerable amounts of fat has been reported.[34] The intravital congo red test was negative.

The extensive involvement of joints and of bones is of especial interest. Masses of amyloid replaced the articular capsules and ligaments of the joints and extended into the joint cavities and intracapsular portions of bone. This together with the involvement of tendons resulted in stiffness and partial ankylosis of the joints, with limitation of motion. The invasion of the left femur led to destruction of the entire neck of the bone and pathologic fracture. Although this fracture was complete and resulted in separation of the head of the femur, the mass of amyloid replacing the bone was so extensive and rigid that the patient was later able to resume slight bearing of weight.

Involvement of tendons and joints in primary systemic amyloidosis has been reported previously by Beneke,[8d] Lubarsch [3a] and von Bonsdorff.[8m] In the cases of Gottron [8j] and Michelson and Lynch [8o] there were arthritic symptoms which suggested joint involvement, but no autopsies were performed. In a case of Beneke's [8d] of primary local amyloid disease of the heart there was, in addition, diffuse involvement by amyloid of tendons and of the ligaments and capsules of the joints. In the first of Lubarsch's [3a] 3 cases there were amyloid deposits in the

33. von Bonsdorff.[8m] Strauss.[8n] Reimann and others.[8r]

34. Lubarsch.[3a] Gottron.[8j] Mollow and Lebell.[8l]

capsules of the knee and hip joints and in the adjacent tendons. Von Bonsdorff's [8m] case is similar to our own. The articular capsules of the shoulder and elbow joints were replaced by amyloid, which invaded the joint spaces and by direct pressure eroded the head and lesser tubercle of the humerus and the olecranon of the ulna. This destruction of bone was demonstrated in roentgenograms during life and at autopsy. Amyloid tumors of the joints have been reported in association with multiple myeloma.[35]

Primary amyloid disease of bone is rare. In generalized secondary amyloidosis bone is usually uninvolved, although there may be infiltration of the small vessels of the marrow. More common are secondary deposits of amyloid in bone or bone marrow the seat of chronic disease or within sarcomas [36] or blastomas of bone, especially multiple myeloma.[37] The present case and that of von Bonsdorff [8m] are the only instances of bone involvement in primary systemic amyloidosis. Solitary primary amyloid tumors of bone involving the sixth and ninth right ribs have been recorded by Edens [2e] and Hedrén,[38] respectively. In Edens' case there was also involvement of the liver, spleen, kidneys, stomach and intestine. Mandl [39] described a case of isolated primary amyloid tumor of the third thoracic vertebra with collapse of the bone. Pressure on the spinal cord produced transverse myelitis. Gerber [2c] recently reported a case of atypical primary amyloid disease involving the liver, spleen and kidneys and diffuse amyloidosis of the bone marrow associated with collapse of the ninth thoracic and first lumbar vertebrae. In all these cases the amyloid was deposited in the reticulum and vessels of the marrow, and the destruction of bone was probably caused secondarily by the pressure of the infiltrating amyloid. In Mandl's [39] case, however, ischemia may have been a factor since the amyloid involvement of the small arteries resulted in virtual occlusion of their lumens. In none of the cases was there reactive formation of new bone.

Involvement of the cardiac valves in primary systemic amyloidosis is unusual, having been described previously only by Wild.[8a] In his case the only information available is that the valve leaflets were

35. Buch, H.: Ein Fall von multipler primärer Sarcomatose des Knochenmarkes, und eine eigenthümliche Affection der vier grossen Gelenke, Med. Dissert., Halle, Lipke, 1873; cited by Magnus-Levy.[31] Zeehuisen, H.: Nederl. tijdschr. v. geneesk. **29**:829, 1893; cited by Magnus-Levy.[31] Hueter, C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:101, 1910. Paige, B. H.: Am. J. Path. **7**:691, 1931.

36. Hildebrand, O.: Virchows Arch. f. path. Anat. **140**:249, 1895.

37. Rosenblum, A. H., and Kirshbaum, J. D.: J. A. M. A. **106**:988, 1936. Freund, E.: Frankfurt. Ztschr. f. Path. **40**:400, 1930. Magnus-Levy.[31]

38. Hedrén, G.: Ztschr. f. klin. Med. **63**:212, 1907.

39. Mandl, J.: Virchows Arch. f. path. Anat. **253**:639, 1924.

thickened as a result of extensive amyloid disease. In Israel's [40] case amyloidosis involved the mitral, tricuspid and pulmonic valves as well as the heart, mouth, larynx, skin and mediastinum, but the deposition of amyloid may have been due to cirrhosis of the liver. Involvement of the valves in isolated primary amyloid disease of the heart is usually slight,[41] although in Koller's [42] case the mitral and tricuspid valves were the seat of extensive deposits. In describing the experimental amyloidosis produced in mice by injections of sodium caseinate Jaffé [43] mentioned lesions of the cardiac valves. The involvement was found mainly in the mitral leaflets, where the amyloid was deposited as a compact layer near the auricular surface. In the present case the severe amyloid involvement of the valves resulted in deformity and circulatory changes. The aortic valve presented thick, shortened cusps, so completely immobile as to preclude their ability to flap or to approximate in diastole. The mitral leaflets were likewise thickened, stiff and immobile. Both valves presented rigid stenotic orifices. The severe passive hyperemia of the lungs was comparable to that seen with chronic rheumatic mitral stenosis. There were hypertrophy and dilatation of the right ventricle, passive hyperemia of the liver and other viscera and hypertrophy of the left ventricle, which was attributed to the aortic valvular disease. The amount of amyloid deposited in the ventricles was not sufficiently great to contribute to their hypertrophy. Examination of the heart failed to reveal evidence of healed rheumatic disease. Numerous microscopic sections of the free portions of the cardiac valves showed considerable deposit of amyloid, but none was found in the blood vessels. Elsewhere in the body, even in such relatively avascular structures as tendons and the ligaments of joints, the small blood vessels in the neighborhood of amyloid nodules, especially arteries, almost invariably showed amyloid infiltration. The extensive involvement of the leaflets may perhaps be explained by direct extension of amyloid from the ring of the valve.

The origin of amyloid in both the primary and the secondary form of amyloidosis is still obscure. Little is known of the nature of amyloid other than what is known of its protein content, homogeneous character and staining reactions. The variable staining of the material in the primary disease as contrasted with the uniform staining in the secondary form indicates that the two types are not identical. The material

40. Israel, I.: Ein Fall von lokalem Amyloid, Med. Dissert., Tübingen, Bochum-Langendreer, 1933.

41. (*a*) Budd, J. W.: Am. J. Path. **10**:299, 1934. (*b*) Larsen, R. M.: ibid. **6**:147, 1930.

42. Koller, F.: Schweiz. med. Wchnschr. **13**:522, 1932.

43. Jaffé, R. H.: Arch. Path. **1**:25, 1926.

recognized histologically as amyloid probably has a variable chemical composition and comprises a group of closely related substances.

The pathogenesis of primary systemic amyloidosis is unknown. Whether the amyloid is the product of infiltration or of degeneration is not clear. The regularity with which the substance is restricted to muscle and connective tissue is significant. Warren [8b] pointed out that since amyloid may be formed in connective tissue at a considerable distance from blood vessels, the substance is the product of abnormal fibroblastic activity. Reimann, Koucky and Eklund [8r] suggested a generalized metabolic perversion of tissue of mesodermal origin. Larsen,[41b] however, in a study of primary myocardial amyloidosis concluded that amyloid is always deposited primarily about venocapillary endothelium. By means of serial sections he established the continuity of nodules of amyloid in isolated areas with deposits around the endothelium of vessels.

Letterer [44] expressed the belief that an etiologic relationship exists between amyloidosis and hyperglobulinemia. There is experimental evidence to support this idea. Reimann and Eklund [45] found that increase in blood globulin regularly accompanied the amyloidosis which they produced in rabbits by injections of sodium caseinate. Dick and Leiter [46] also noted hyperglobulinemia in association with the amyloidosis produced in rabbits by the use of various strains of streptococci. Amyloid disease occurs frequently in horses used to produce various antiserums,[47] and such animals show an increase, often marked, in serum globulin.[48] Clinically, only a few determinations of blood protein have been made in cases of primary systemic amyloidosis, and these were within normal limits.[49] Hyperglobulinemia is rare with secondary amyloidosis. It has been reported in cases of multiple myeloma, in which the increase in plasma protein was almost entirely in the euglobulin fraction.[50] The experimental findings suggest that amyloid may represent a reaction between some component of the serum globulin and certain fixed tissue elements which in the case of primary amyloidosis are present in mesoblastic structures or in the walls of

44. Letterer, E.: Beitr. z. path. Anat. u. z. allg. Path. **75**:486, 1926.

45. Reimann, H. A., and Eklund, C. M.: Am. J. M. Sc. **190**:88, 1935.

46. Dick, G. F., and Leiter, L.: Tr. A. Am. Physicians **52**:246, 1937.

47. Doerken, E.: Virchows Arch. f. path. Anat. **286**:487, 1932. Arndt, H. J., and Doerken, E.: Arch. f. wissensch. u. prakt. Tierh. **63**:1, 1931.

48. Reitstötter, J.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **30**:468, 1920.

49. Reimann and others.[8r] DeNavasquez and Treble.[8b]

50. Perlzweig, W. A.; Delrue, G., and Geschickter, C.: J. A. M. A. **90**: 755, 1928. Shirer, J. W.; Duncan, W., and Haden, R. L.: Arch. Int. Med. **50**:829, 1932.

capillaries or arterioles. Such a reaction may possibly be of antigen-antibody nature and may have an allergic basis.

SUMMARY

A case of primary systemic amyloidosis is reported. The duration of the disease was fourteen years. There was extensive involvement of the joints and of bones, with pathologic fracture of the left femur, and there was extensive amyloid infiltration of the valves of the heart.

Primary systemic amyloidosis, although rare, has become a recognized entity. There are now in the literature reports of 23 cases. The disease is apparently primary and is characterized by a general distribution of amyloid in mesodermal structures of the body, especially in smooth and skeletal muscle. The most frequently affected organs are the tongue, heart, stomach, intestine and skeletal muscles. The liver, spleen and kidney, usually affected in the secondary form of amyloidosis, are rarely involved.

The genesis of the disease is obscure. Whether the amyloid is the result of infiltration or of degeneration of mesodermal tissue is not clear. The atypical staining of the amyloid in the primary form as contrasted with the uniform staining in secondary amyloidosis suggests that the two types are not identical. The material recognized histologically as amyloid probably comprises a group of substances closely related in chemical structure.

SENSITIZATION, ANTIBODY FORMATION AND LESIONS PRODUCED BY TUBERCLE BACILLI IN THE ALBINO RAT

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Experimental tuberculosis has been studied mainly in the guinea pig and rabbit. The former species is used because it contracts tuberculosis with little resistance, and the latter, because it is highly susceptible to infection by the bovine type of tubercle bacilli. It seems desirable to approach some questions of pathogenesis and immunity through studies in a highly resistant species, the albino rat. Tuberculosis in the rat may offer opportunities to correlate some aspects of the disease under conditions not observed in the highly susceptible species of animals.

In general, the rat stands apart from man and many lower animals in regard to phenomena of hypersensitiveness and antibody formation. Anaphylaxis is demonstrable with difficulty in the rat (Longcope [1]; Parker and Parker [2]) and the Arthus phenomenon cannot be induced (Longcope [1]; Opie [3]). Neither has the tuberculin skin reaction or the Koch phenomenon been elicited in this species (Boquet and Nègre; [4] Gloyne and Page; [5] Ornstein and Steinbach; [6] Boquet, Nègre and Valtis; [7] M. I. Smith; [8] W. Jadassohn [9]).

Some workers have attempted to correlate the allergic reactivity of the skin of the guinea pig and of the rabbit with tubercle formation and caseation. The lack of skin reactivity in the rat when studied in conjunction with the pathogenesis of tuberculosis may throw additional

From the Department of Pathology, Cornell University Medical College.

1. Longcope, W.: J. Exper. Med. **36**:627, 1922.
2. Parker, J. T., and Parker, F., Jr.: J. M. Research **44**:263, 1924.
3. Opie, E. L.: J. Immunol. **9**:231, 1924.
4. Boquet, A., and Nègre, L.: Ann. Inst. Pasteur **35**:142, 1921.
5. Gloyne, S. R., and Page, D. S.: J. Path. & Bact. **26**:224, 1923.
6. Ornstein, G. G., and Steinbach, M. M.: Am. Rev. Tuberc. **12**:77, 1925.
7. Boquet, A.; Nègre, L., and Valtis, J.: Compt. rend. Soc. de biol. **97**:1665, 1927.
8. Smith, M. I.: Pub. Health Rep. **43**:2817, 1928.
9. Jadassohn, W.: Arch. f. Dermat. u. Syph. **167**:169, 1933.

light on the possible relationship of allergy to some aspect of the disease, for instance, caseation.

Many attempts have been made to produce "tuberculin death" in the rat. Most of them have failed (Boquet and Nègre;[4] Gloyne and Page;[5] Ornstein and Steinbach;[6] Boquet, Nègre and Valtis;[7] Schütze and Zilva[10]). Smith and Hendrick[11] found that the intraperitoneal injection of 0.5 cc. of tuberculin killed few tuberculous rats when they were kept on a normal diet but killed most of them when they were fed on a diet deficient in vitamin A. More recently, M. I. Smith[8] studied the effect of an intravenous injection of a filtrate (protein) from cultures of tubercle bacilli on tuberculous rats. He found that 10 mg. of protein killed 1 of 12, 50 mg. 6 of 10 and 100 mg. all of 10 tuberculous rats. All normal rats were resistant to 200 mg., but 3 of 5 died after an injection of 300 mg. Lack of vitamin A made both normal and tuberculous rats more susceptible to the lethal effect of tuberculoprotein. Rats submitted to intraperitoneal injection of heat-killed tubercle bacilli were not sensitive to tuberculin.

The rat seems to be an unusual species in regard to antibody formation. The production of antibodies against either toxins or foreign proteins is scant (Coca and others;[12] Longcope;[1] Parker and Parker[2]). Ornstein and Steinbach[6] reported that rats infected with tubercle bacilli do not produce antibodies.

The first systematic investigation of the pathogenesis of tuberculosis in the rat was made by the British Royal Commission on Tuberculosis.[13] They concluded that the rat is highly resistant to infection with tubercle bacilli and that infection in the rat differs conspicuously from that in man and susceptible lower animals. The commission found that when the rat is infected subcutaneously with tubercle bacilli or is fed tubercle bacilli it fails to present generalized tuberculosis. After intraperitoneal or intravenous injection of very large doses of human or bovine tubercle bacilli rats may die with the Yersin[14] type of tuberculosis, which is characterized by abundant multiplication of organisms in the tissues in the absence of tubercles. Gloyne and Page,[5] using rats, injected 1,000,000,000 human tubercle bacilli into the subcutaneous tissue, peritoneal cavity or testis and observed the results for forty-five days.

10. Schütze, H., and Zilva, S. S.: J. Hyg. **26**:204, 1927.

11. Smith, M. I., and Hendrick, E. G.: J. Lab. & Clin. Med. **11**:712, 1926.

12. Coca, A.; Russell, E. F., and Baughmann, W. H.: J. Immunol. **6**:387, 1921.

13. Report of the Royal Commission on Tuberculosis (1907-1911), cited by Cobbett, L.: The Causes of Tuberculosis, Cambridge, University Press, 1917, p. 443; cited by Gloyne and Page;[5] cited by Griffith, A. S.: Experimental Tuberculosis, in A System of Bacteriology, Privy Council, Medical Research Council, London, His Majesty's Stationery Office, 1930, vol. 5, p. 169.

14. Yersin, M. A.: Ann. Inst. Pasteur **2**:245, 1888.

Macroscopic tubercles were found in the lungs of only a few animals. Microscopic lesions were seen in the lungs, spleen, liver, lymph nodes and bone marrow. These comprised poorly stained collections of phagocytes with tubercle bacilli in and around them. Lange [15] found that rats which received approximately 1,500,000 bovine tubercle bacilli into the subcutaneous tissue acquired histologic tubercles at the sites of injection and in the draining lymph nodes. The tubercles and tubercle bacilli gradually disappeared. Ornstein and Steinbach [6] gave rats an intraperitoneal injection of 0.33 mg. of a human strain of low virulence (H37). The animals were killed from three to ninety-three days after infection. Macroscopic or microscopic tubercles were not found, but tubercle bacilli were present in smears and sections of many organs. The authors were able to infect guinea pigs with the rat tissues. More recently Steinbach [16] has confirmed and extended these observations. Rats that received 1 mg. of human (H37) or bovine (B1) tubercle bacilli intraperitoneally had no macroscopic or microscopic tuberculous lesions from twenty-five to two hundred and six days after inoculation, although the bacilli were found in smears and histologic sections of the viscera. Rats subjected to adrenalectomy or thyroparathyroidectomy and similarly infected with bovine tubercle bacilli of the B1 strain had macroscopic and microscopic tubercles of the spleen, liver, omentum, retroperitoneal lymph nodes and diaphragm within two months after infection. Caseation was often observed.

Smith and Hendrick [11] studied tuberculous lesions in rats given an intraperitoneal injection of 5 mg. of a human strain of tubercle bacilli of low virulence (H37). They observed large multinucleated cells with eosinophilic cytoplasm and occasional giant cells in the omentum, spleen, liver and lymph nodes, as well as aggregates composed of epithelioid cells, large multinucleated epithelioid cells, occasional giant cells and lymphocytes in the lungs. Caseation was never observed, but intracellular lipoid globules were found in the epithelioid cells in the lungs. Large numbers of bacilli were found in the lungs of rats dying one year after infection. The other organs contained few tubercle bacilli. Long and Vorwald [17] and Vorwald [18] examined histologic sections of lungs of rats six months after an intravenous injection of approximately 0.01 mg. of human tubercle bacilli (H37). They found typical tubercles, containing tubercle bacilli, with no caseation.

The plan of the present work was to study the cutaneous sensitiveness, the systemic reaction to tuberculin and the production of comple-

15. Lange, L. B.: Am. Rev. Tuberc. **7**:49, 1923; **11**:241, 1925; **15**:629, 1927.
16. Steinbach, M.: Am. Rev. Tuberc. **26**:52, 1932.
17. Long, E. R., and Vorwald, A. J.: Nat. Tuberc. A. Tr. **26**:205, 1930.
18. Vorwald, A. J.: Am. Rev. Tuberc. **27**:270, 1933.

ment-fixing antibodies in rats after an injection of heat-killed tubercle bacilli or after infection. The pathogenesis of tuberculosis was studied in normal and immunized animals.

MATERIAL AND METHODS

Mature male albino rats weighing from 130 to 170 Gm. were used. The diet was a mixture of hominy, sodium chloride, rolled oats, meat scraps, skimmed milk and wheat, which was fed four times a week; a mixture of lettuce and cauliflower or broccoli stems was fed three times a week; and dog biscuits [18a] were given twice a week. Rats have been maintained on this diet for several years and have not shown signs of avitaminosis.

The tubercle bacilli used were of the bovine type, strain Ravenel, isolated more than twenty years ago by Dr. M. Ravenel. This strain possesses high virulence, so that 0.00001 mg. injected intravenously kills rabbits with extensive pulmonary and renal tuberculosis in from three to six months. Rats were infected with 1 mg. and occasionally 10 mg. of this strain. Injections were made through a 29 gage hypodermic needle into the exposed left femoral vein.

A suspension of killed tubercle bacilli was employed both as an immunizing agent and as antigen for complement fixation. It was prepared in the following manner: Weighed amounts of bacillary growth on glycerin agar, approximately five weeks old, were ground and suspended in saline solution; 1 cc. of the suspension contained 10 mg. The suspension was heated in an Arnold steam box for half an hour at 100 C. and preserved with 0.35 per cent cresol, U. S. P.

Tuberculin prepared from cultures of the Ravenel strain was approximately one and a half times as potent as the international standard tuberculin. As a rule, 0.1 cc. of a 1 in 5 dilution was injected into the skin. Varying quantities of the same dilution were used for intraperitoneal injection.

Complement fixation was employed to determine the antibody titer of the blood. Fresh rat serums were inactivated by heating at 55 C. for thirty minutes. Approximately one fourth of the self-inhibiting dose of the antigen was used in the complement fixation. The complement, namely, guinea pig serum, was always freshly titrated, and 2½ units was used. For the test 0.25 cc. of each of the aforementioned ingredients was incubated in a water bath at 37 C. for one hour. Then 0.25 cc. of a suspension of washed sheep cells (in one twentieth of their concentration in whole blood) and 0.25 cc., or 2 units, of antisheep hemolysin was added. The whole system was then incubated at 37 C. until the standard serum indicated completion of the second phase. To insure the accuracy of the test, a standard serum was always included. This was obtained from a rabbit that had been immunized by a series of injections of tubercle bacilli and was preserved in the

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18a. Purima Dog Chow contains:

Wheat germ	Dried meat
Barley	Molasses
Carotine (vegetable compound)	Corn grit
Malt	Cereal feed
Dried beef pulp	Cod liver oil
Dried skimmed milk	Iodized salt
Brewers' dried yeast	

Protein, 20 per cent; fat, 6 per cent; fiber, 6 per cent, and nitrogen-free extract, 46 per cent.

dried state in 0.5 cc. portions according to the method of Elser, Thomas and Steffen [19] or that of Flosdorf and Mudd.[20] Each sample of dried serum was recovered as needed by the addition of 0.5 cc. of distilled water. That the method of drying yielded uniform samples was shown by testing 3 samples of dried serum simultaneously. The variation, if any, in the complement fixation tests over a period of months was slight, since the highest dilution of the standard serum that fixed complement was always the same.

The methods used in the immunization of rats with heat-killed tubercle bacilli were as follows: The animals of group A received a weekly intracutaneous injection of 0.1 mg. of heat-killed tubercle bacilli, respectively, for eight weeks. The rats in group B received, in all, seven intracutaneous injections of 0.2 mg. of heat-killed tubercle bacilli, given at four day intervals. The animals of group C were each given seven simultaneous subcutaneous injections of 0.2 mg. of heat-killed tubercle bacilli, followed by weekly subcutaneous injections of 0.2 mg. each

TABLE 1.—*Tuberculin Reactions in Normal and Tuberculous Rats*

Group	Tuberculin		Rat	Days After Infection	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
	Mg.	Route					
Normal	20	Intracutaneous	8, 9	....	0	0	0
	100	Subcutaneous	58, 59, 61, 63	....	0	0	0
	800	Intraperitoneal	51	....	..	0	0
	1,000	Intraperitoneal	52	....	..	0	0
Infected	20	Intracutaneous	16	13, 29	0	0	0
			11, 17	13, 29, 46	0	0	0
			30	13, 29, 46, 70	0	0	0
			11	70	0	+	In 2 days
			20	138	0	+	0
			23	145	0	+	In 2 days
			22*	145	0	+	Within 1 day
			51, 52, 54	164	0	+	0
			56	175	0	+	0
	20	Intraperitoneal	51, 52	34, 84	..	+	0
			57	34	..	+	Within 1 day
			54	84	..	+	0
			56	95	..	+	0
	100	Intraperitoneal	53	14	..	+	Within 1 day
			24, 25	96	..	+	Within 1 day

* This rat was infected with 10 mg. of tubercle bacilli, injected intravenously.

for four weeks. Some of these animals (constituting group D) received one or two intraperitoneal injections of 0.2 mg. of heat-killed bacilli within twenty-one days after the last subcutaneous injections.

EXPERIMENTAL OBSERVATIONS

Hypersensitiveness.—Ten tuberculous rats were tested by injecting intracutaneously into each 20 mg. of tuberculin at one or more intervals varying from thirteen to one hundred and seventy-five days after infection. In no instance was a positive skin reaction, i. e., redness and edema, obtained. In contrast to the lack of skin reaction, systemic reactions occurred in some of the rats so treated (table 1). The rats

19. Elser, W. J.; Thomas, R. A., and Steffen, G. I.: J. Immunol. **28**:433, 1935.

20. Flosdorf, E. W., and Mudd, S.: J. Immunol. **29**:389, 1935.

tested thirteen, twenty-nine and forty-six days after infection gave no evidence of hypersensitiveness, but 3 of 4 rats that received 20 mg. of tuberculin intradermally from seventy to one hundred and forty-five days after infection became ill and died within two days after the injection. Four rats that were tested with tuberculin from one hundred and sixty-four to one hundred and seventy-five days after infection became ill, as was indicated by irritability and ruffling of the body hair, but survived the injection. Twenty milligrams of tuberculin was injected into the peritoneal cavity of each of 5 tuberculous animals at one or more intervals varying from thirty-four to ninety-five days after infection. The symptoms of irritability and ruffling of hair followed each injection, but only a single rat died. In a final experiment 1 rat, infected fourteen days previously and 2 rats, infected ninety-six days previously, were given an intraperitoneal injection of 100 mg. of tuberculin. All died within twenty-four hours. These observations suggest that the

TABLE 2.—*Tuberculin Reactions in Rats Immunized with Heat-Killed Tubercle Bacilli*

Tuberculin		Immunization Group	Animals	Days After Immunization	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
Mg.	Route						
20	Intracutaneous	A	5	6	0	0	0
		B	5	4	0	0	0
		C	2	9	0	0	0
1,000	Intraperitoneal	D	1	29	..	+	0
		D	1	29	..	+	In 5 days

site of injection, i. e., the skin or the peritoneal cavity, did not influence the systemic effect of tuberculin. Table 1 also shows that neither cutaneous reaction to nor death from tuberculin occurred in normal rats. As large an amount as 1,000 mg. of tuberculin injected into the peritoneal cavity of a normal rat failed to cause noticeable general reaction.

The injection of heat-killed tubercle bacilli into the skin of normal rats was followed by the formation of nodules from 2 to 4 mm. across and less than 0.5 mm. high at the sites of injection. These nodules did not increase in size or ulcerate. This reaction to the antigen did not change when the injection was repeated. Rats repeatedly given injections of heat-killed tubercle bacilli did not show local reactions to 20 mg. of tuberculin injected into the skin (table 2), and systemic reactions did not follow these injections. On introduction of 1,000 mg. of tuberculin into the peritoneal cavity of each of 2 immunized rats, general malaise developed in both. One died after five days.

In rats first immunized with heat-killed tubercle bacilli and later infected with living tubercle bacilli skin reactions to tuberculin did not occur. However, systemic reactions and death were produced regu-

larly and promptly (table 3). A comparison of tables 1 and 3 suggests that the injection of heat-killed tubercle bacilli prior to infection made the rats more susceptible to the toxic action of tuberculin.

Antibody Formation.—Complement fixation tests were performed on 46 samples of serum from 31 normal rats. From some of the rats samples of blood were obtained on two occasions. Complement fixation was observed regularly with undiluted rat serums and frequently (47 per cent) with serums diluted 1 in 2.5. As a rule, when serums were diluted 1 in 5 fixation did not occur. There were 5 serums that fixed complement in such a dilution (11 per cent). None of the serums reacted in dilutions of 1 in 10, 1 in 20 or 1 in 40.

Complement fixation was tested with the serums of 12 rats infected with 1 mg. or 10 mg. of tubercle bacilli. With 2 rats, tests were made forty-nine, eighty-nine and one hundred and forty-five days after infec-

TABLE 3.—*Tuberculin Reactions in Tuberculous Rats That Had Been Immunized with Heat-Killed Tubercle Bacilli Prior to Infection*

Tuberculin		Rat	Immunization Group	Days After Infection	Skin Reaction	Systemic Reaction	Death from Reaction to Tuberculin
Mg.	Route						
20	Intracutaneous	32	D	25	0	+	0
		33	D	34	0	+	0
		46	D	45	0	+	Within 1 day
		6	A	96	0	+	0
		5, 10, 14	A	96	0	+	Within 1 day
		6	A	171	0	+	Within 1 day
100	Intraperitoneal	31	D	18	..	+	Within 1 day
200	Intraperitoneal	27	D	18	..	+	Within 1 day

tion. The tests were carried out as a rule with serum dilutions from 1 in 5 to 1 in 40. Complement fixation was obtained with 4 serums in 1 in 5 dilution. A serum in this group failed to give complement fixation when retested on two later occasions. With serums diluted higher than 1 in 5, fixation was never observed.

While an increase in complement-fixing antibodies was not observed in rats that had been merely infected with tubercle bacilli, a significant rise in antibody titer was noted in those animals that had received injections of tuberculin in addition to infection (table 4). Of 7 serums from rats that had been infected from one to five months previously with 1 mg. of tubercle bacilli and subsequently given 20 mg. of tuberculin on one or more occasions, 5 fixed complement in a dilution of 1 in 10, and 2 showed fixation in a dilution of 1 in 20. One of these when retested several months later was negative in a dilution of 1 in 10. Two serums from rats that had received large intraperitoneal injections of tuberculin before infection, as well as subsequent small doses, showed the same titers of antibody as the serums of infected animals that had received only small doses of tuberculin. A rat that died following an

intracutaneous injection of 20 mg. of tuberculin gave serum post mortem that failed to fix complement in a dilution of 1 in 5.

The repeated injection of heat-killed tubercle bacilli into normal rats induced formation of antibodies regardless of the mode of immunization (table 5). A higher percentage of the animals immunized by the intracutaneous route (groups A and B) showed increase in antibodies

TABLE 4.—*Antibody Titers of Serums of Tuberculous Rats After Injections of Tuberculin*

Tuberculin			Days Between Infection and Injections of Tuberculin	Days Between Infection and Complement Fixation	
Mg.	Route	Rat			Titer
20	Intracutaneous	16	13, 29	31	10
		11*	13, 29, 46, 70	72	0
		20	13, 29, 46, 70, 138	157	10
20	Intraperitoneal	51, 52	34	48	10
		51, 54	34, 84	98	20
		52	34, 84	98	5
		56	45, 95	109	5

* The blood used was obtained after death from reaction to tuberculin.

TABLE 5.—*Antibody Titers of Serums of Rats Immunized with Heat-Killed Tubercle Bacilli*

Rat	Immunization Group	Days After Immunization	Titer
14			10
5	A	8	20
6			40
M9			0
M1, M5, M8	B	6	5
M3			20
27, 28, 31, 33, 34, 35, 36, 38, 40, 46			0
29, 39	C	6	10
32			40
35, 36			10
33, 38, 40			20
31, 39, 46	D	18	40
27, 32			80
40			20
27	D	30	40
33, 35, 36			20
27, 31	D	36	40
32			80

than of those given subcutaneous injections (group C). The highest antibody titers were found when rats had been given one or two intraperitoneal injections of 0.2 mg. of heat-killed tubercle bacilli subsequent to subcutaneous injections (group D). The serums of 5 of 10 rats so treated fixed complement in a dilution of 1 in 40, and 2 of these serums were positive in a dilution of 1 in 80.

The serums of 7 tuberculous rats that had received a series of intracutaneous injections of heat-killed tubercle bacilli before infection did not fix complement at appreciably higher dilutions than the serums of

nonimmunized tuberculous animals (table 6). Two rats given intracutaneous injections of 20 mg. of tuberculin ninety-six days after infection had high antibody titers.

Tuberculosis in the Rat.—Eight adult male albino rats were infected intravenously with 1 mg. of the Ravenel strain of bovine tubercle bacilli. Three died between twenty-six and eighty-seven days after infection. Two died six months after infection. One died ten months after, another one year after, while still another survived eighteen months. It is uncertain to what extent tuberculosis shortened the normal life expectancy of these animals, since we had no control series of noninfected animals. Many of the rats had extensive nontuberculous pulmonary lesions, i. e., bronchiectasis and abscesses, that evidently preceded tuberculous infection. The only gross lesions of tuberculosis induced in these rats were minute tubercles in the lungs and enlargement of the spleen. The great resistance of the rat to infection with

TABLE 6.—*Antibody Titers of Serums of Tuberculous Rats That Had Been Immunized with Heat-Killed Tubercle Bacilli Previous to Infection*

Immunization Group	Rat	Days After Infection	Titer
A	5, 14	75	0
	6	75	5
	14*	97	40
	6*	115	20
B	M9	29	0
	M8	35	5
	M1	42	5
	M5	49	0

* In this rat 20 mg. of tuberculin had been injected intracutaneously ninety-six days after infection.

tubercle bacilli is apparent; for, if rabbits are infected intravenously with 0.00001 mg. of the same strain, they succumb within from three to six months and at autopsy present massive tuberculosis of the lungs and kidneys (Opie and Freund [21]).

Since the pathogenesis of tuberculosis in rats has been inadequately described and is a subject of controversy, a description of the pathologic changes in the animals observed in this study is desirable. Postmortem examinations were made of 26 rats that died or were killed from two hours to eighteen months after the intravenous injection of 1 mg. of bovine tubercle bacilli. Parts of the lungs, spleen, liver and kidneys were fixed in Zenker's fluid, embedded in paraffin and cut 6 microns in thickness. Duplicate sections were stained with hematoxylin and eosin and by the Ziehl-Neelsen method. The fate of the injected tubercle bacilli was followed by counting the number of acid-fast rods in 100 oil immersion fields (Zeiss, × 900) of the sections stained by the Ziehl-Neelsen method.

21. Opie, E. L., and Freund, J.: J. Exper. Med. **66**:761, 1937.

Lung: The lungs of all animals infected more than one month previously showed minute grayish white nodules, discrete and confluent, uniformly distributed over their pleural and cut surfaces (fig. 1 *A*). These gross tuberculous lesions were differentiated from the spontaneous abscesses of the lungs found in many rats by their small size, gray color and uniform distribution. Histologic examination revealed tuberculous lesions in various stages of development in all of the animals.

Two hours after infection, clumps of acid-fast bacilli were found in the alveolar capillaries in the subpleural region, and occasional bacilli were seen in single polymorphonuclear leukocytes or large mononuclear cells in the alveolar septums. From three to fifteen days after infection, tubercle bacilli were found in round collections of large mononuclear cells in the alveolar septums. After the second week, these simple tubercles, measuring 20 to 60 microns in diameter, changed little in size and were often intra-alveolar. The cells that formed them had the character of epithelioid cells and often contained acid-fast bacilli. In them, also, were granules that assumed a brown color but were not acid-fast by the Ziehl-Neelsen method. The cells had light staining oval or irregularly elongated nuclei, containing a few coarse chromatin granules, a prominent nucleolus and a heavy folded nuclear membrane. Their cytoplasm was abundant, lightly acidophilic and reticulated, and the cell boundaries were often indistinct. Single necrotic epithelioid cells were seen in many of the tubercles, but caseation was never observed. Occasional giant cells of the Langhans type, containing bacilli and granules, were found within the pulmonary alveoli of most animals after the second week.

The minute tubercles that we saw on the surfaces of the lungs of all rats after one month (fig. 1 *A*) were subpleural groups of epithelioid cells, in part within the septums and in part intra-alveolar; i. e., associated with a small tubercle there was a subpleural focus of tuberculous pneumonia (fig. 1 *B*).

Table 7 shows the number of acid-fast rods demonstrable in 100 oil immersion fields in sections of lung. Two hours after infection, approximately 200 bacilli per hundred fields were demonstrable. Fewer tubercle bacilli were found in 14 animals that were killed within seventy-two days after infection, although an animal that died twenty-six days after infection had a very large number of organisms in the lungs. After seventy-three days, the figures varied but were on the whole higher than in the earlier period. In 3 animals that died six, twelve and eighteen months after infection the number of tubercle bacilli in the lungs was high, i. e., several thousand per hundred oil immersion fields.

The lesions in animals that had tubercle bacilli in great numbers in their lungs were similar to those in the other rats. Caseation was

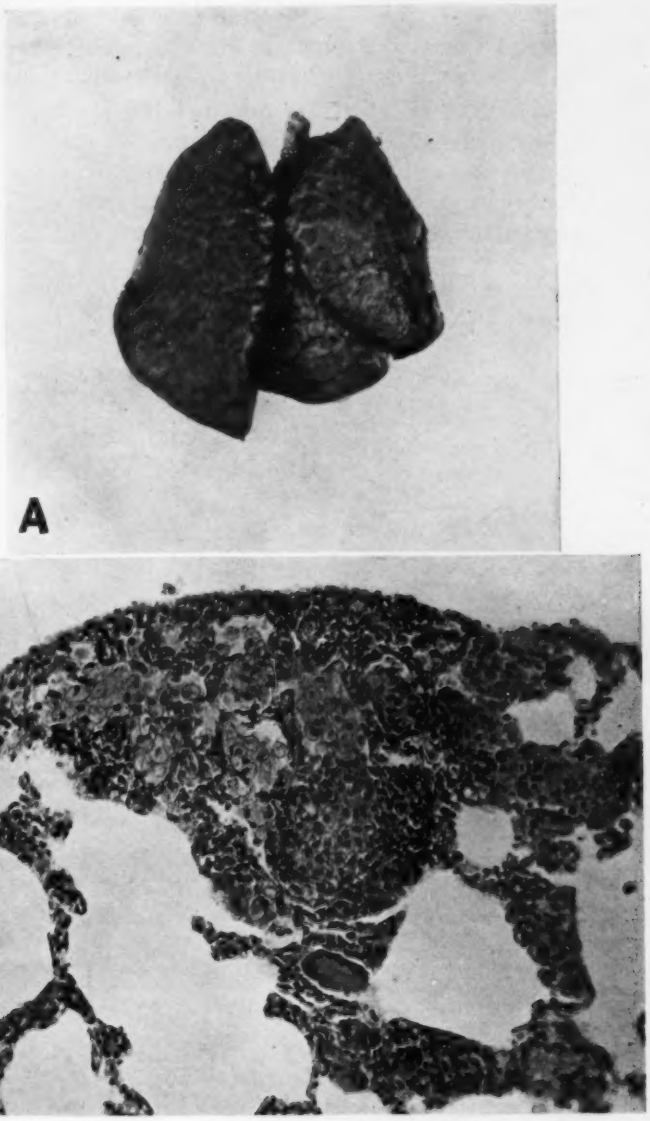

Fig. 1.—*A*, posterior aspect of the lungs of rat 51 which died ten months after an intravenous infection with 1 mg. of bovine tubercle bacilli. Note the discrete and confluent tubercles distributed over the surfaces of these lungs (natural size). *B*, section through a tubercle below the surface of the lung of rat M12, which was killed forty-nine days after infection; × 200. The tubercle is a group of septal and intra-alveolar collections of epithelioid cells in which tubercle bacilli are found. Note the Langhans giant cell.

absent, and the number of epithelioid cells with necrosis was small; tubercle bacilli were seen in groups in small tubercles composed of epithelioid cells and among the peripherally placed nuclei of giant cells. Granules which were not acid-fast were seen within epithelioid cells of many of the tubercles after one week and were regarded as presumptive evidence of destruction of tubercle bacilli. Nevertheless, the occurrence of well preserved and viable organisms long after infection indicates that destruction was not complete. Numerous acid-fast bacilli were seen in epithelioid cells and among the peripherally placed nuclei of giant cells in the lungs of a rat eighteen months after infection

TABLE 7.—*Tubercle Bacilli in One Hundred Oil Immersion Fields of Organs of Rats Infected Intravenously with 1 Mg. of Bovine Tubercle Bacilli*

Rat	Time After Infection	Tubercle Bacilli Counted		
		Lung	Spleen	Liver
43	2 hours	272	19	0
41	2 hours	177	13	0
42	3 days	79	1	1
44	8 days	80	57	47
45	10 days	30	2	24
15	15 days	4	19	14
53*	15 days	10	4	12
M13	19 days	26	12	47
26†	26 days	About 5,000	335	143
M14	29 days	0	6	0
16	31 days	1	14	19
57*	35 days	21	14	6
M10	35 days	0	0	0
M11	42 days	0	0	0
M12	49 days	1	0	0
17	62 days	23	0	0
11*	72 days	48	6	12
8†	73 days	242	0	0
7†	87 days	0	0	0
25*	97 days	..	0	0
23*	147 days	124	12	0
56†	181 days	303	83	13
20†	186 days	About 2,000	0	0
51†	306 days	131	0	8
52†	352 days	About 2,000	..	0
54†	477 days	About 5,000	201	40

* Death was due to the reaction to tuberculin.
† The rat died.

(fig. 2*A*). Lung tissue from this animal, seeded on Petragnani's medium, yielded abundant growth of tubercle bacilli within one month.

Spleen: Although macroscopic tubercles were never present in the spleen, the organ was enlarged, and microscopic tubercles were present in it in all animals after the first week. Two hours after infection, a few clumps of acid-fast bacilli were found in the terminal arterioles of the pulp, and occasionally acid-fast bacilli were seen in polymorphonuclear or mononuclear cells in the cords of the pulp. From the end of the first week to the end of the fifth week, numerous tubercle bacilli were found in many discrete or rarely confluent groups of epithelioid cells, measuring from 20 to 40 microns across, and situated in the splenic corpuscles and in the pulp cords (fig. 2*B*). Single necrotic

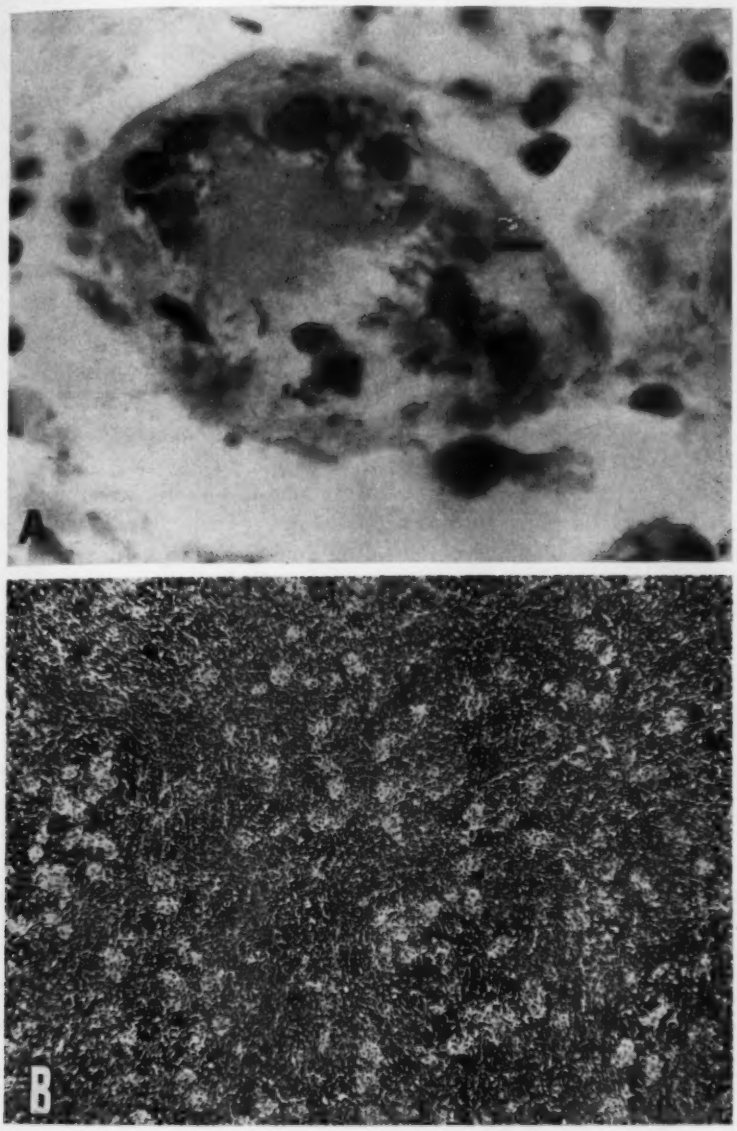

Fig. 2.—*A*, giant cell in the lung of rat 54, which died eighteen months after infection; × 1,700. Numerous tubercle bacilli may be seen among the peripherally placed nuclei. *B*, spleen of rat 44, which was killed eight days after infection; × 62. Numerous small discrete nodules composed of epithelioid cells that contain tubercle bacilli are seen in the red and white pulp. The spleen weighed 2 Gm.

epithelioid cells were seen in many of the tubercles, but caseation was not observed. Many megakaryocytes, as well as numerous mononuclear cells in mitosis, were seen in the splenic pulp. The spleens averaged in weight approximately 2 Gm., the weight of the spleen of a normal rat being approximately 0.5 Gm. From the sixth week to the tenth month, tubercle bacilli were found with difficulty in most of the spleens but were abundant in some. The small collections of epithelioid cells noted in a foregoing statement were slightly reduced in size. Giant cells and fibroblasts were rarely seen. The spleen in this period varied in weight from 0.8 to 2.4 Gm.

From the time of infection up to thirty-five days, tubercle bacilli were demonstrable in all spleens (table 7); after this time in most spleens none was observed. Nevertheless, in animals with exceptionally large numbers of tubercle bacilli in the lungs, the organisms were demonstrable in the spleen. It is noteworthy that yellowish brown lipoid droplets, as well as nonacid-fast granules, occasionally in chains the length of tubercle bacilli, were observed in epithelioid cells of many spleens after the first month.

Liver: Macroscopic tubercles were not found in the liver. Small microscopic collections of epithelioid cells, often containing tubercle bacilli, were seen in the livers of all animals after eight days and were similar to those found in sections of lung and spleen. These simple tubercles occurred within the hepatic lobule and in periportal spaces; their number and size diminished with increasing intervals after infection. After corresponding intervals they were less numerous in the liver than in the lung or spleen. Giant cells were found in the liver of a single animal which was killed two weeks after infection.

Two hours after the injection of 1 mg. of bovine tubercle bacilli, no organisms could be demonstrated in the liver; from three to thirty-five days after infection, they were present in most of the animals. After thirty-five days, they were found only in those rats the lungs of which had shown the micro-organisms in great numbers. Nonacid-fast granules were seen in tubercles of the liver in 2 rats one month and six months after infection.

Kidney: Gross lesions were not found in the kidney. The characteristic tubercles composed of epithelioid cells were seen in the kidneys of 2 rats that were killed ten and thirty-five days after infection. Tubercle bacilli were found in scattered large mononuclear cells in the interstitial tissue of the kidney of a single rat eighteen months after infection.

Tuberculosis in Rats Previously Treated with Heat-Killed Tubercle Bacilli.—Autopsies were made on 13 rats that had been given repeated injections of heat-killed tubercle bacilli and subsequently infected intravenously with 1 mg. of bovine tubercle bacilli. These animals died or were killed from nineteen to one hundred and seventy-two days after

infection. Minute macroscopic tubercles were found in the lungs of all the animals after one month, and conspicuous enlargement of the spleen occurred in every rat. There were histologic tubercles composed of epithelioid cells in the lungs, spleen and liver of every animal. Occasional necrotic epithelioid cells were present in many of the tubercles, but caseation was never observed. Giant cells were seen in the lungs of most rats but were found in the spleens or livers of only a few. The lesions were similar in every respect to those of the tuberculous rats described.

Tubercle bacilli were present in small numbers in the lungs, spleen or liver in 5 immunized and infected rats, and none were found in the organs of 8 animals (table 8). A comparison of tables 7 and 8 shows that fewer tubercle bacilli were demonstrable in the organs of the

TABLE 8.—*Tubercle Bacilli in One Hundred Oil Immersion Fields of Organs of Immunized Rats Infected Intravenously with 1 Mg. of Bovine Tubercle Bacilli*

Rat	Immunization Group	Time After Infection, Days	Tubercle Bacilli Counted		
			Lung	Spleen	Liver
27*	D	19	0	0	0
31*	D	19	0	0	0
M3	B	19	6	14	18
M9	B	29	0	0	0
M8	B	35	0	0	0
M1	B	42	0	0	0
M5	B	49	0	0	0
13†	A	61	53	0	0
2†	A	74	4	19	0
5*	A	97	2	0	0
10*	A	97	0	0	0
14*	A	97	0	0	0
6*	A	172	90	6	3

* This rat died of the reaction to tuberculin.
† This rat died.

immunized than in the organs of the previously normal rats. Organisms were seen in the lungs, spleen or liver in 11 of 15 previously normal rats that were examined between nineteen and one hundred and eighty-one days after infection (table 7). Nonacid-fast granules were present in epithelioid cells in the lungs of all immunized and infected rats and in the spleens or livers of only a few animals.

COMMENT

The experiments reported in the present paper show that in rats in which tuberculosis developed following the intravenous injection of 1 or 10 mg. of virulent bovine tubercle bacilli the skin did not react to tuberculin. Skin reaction to tuberculin also failed to occur after repeated injections of killed tubercle bacilli, even if this treatment was followed by infection with bovine tubercle bacilli. The small nodules that followed injections of heat-killed tubercle bacilli did not increase

in size with repeated injections, as in the rabbit (Opie and Freund [21]). Tuberculin repeatedly injected into infected rats did not induce cutaneous sensitiveness to tuberculin.

In contrast to the lack of skin reaction to tuberculin, there was systemic reaction in infected rats. Normal rats tolerated 1,000 mg. of tuberculin injected into the peritoneal cavity. Tuberculous rats died after the injection of 20 mg. of tuberculin. It was noteworthy that the site of injection of tuberculin, namely, the skin or the peritoneal cavity, did not seem to influence the systemic reaction. That the rat possesses a high degree of hypersensitiveness is further indicated by the experiments of Freund,[22] who found that tuberculous guinea pigs succumb to the intraperitoneal injection of 120 mg. of tuberculin but not to 60 mg. One thousand milligrams of tuberculin injected into 2 rats immunized with heat-killed tubercle bacilli caused general malaise in both. One of these animals died after five days.

Our observations in regard to the systemic effect of tuberculin on tuberculous rats differ from those recorded in the literature. All previous attempts to demonstrate systemic hypersensitiveness to tuberculin in tuberculous rats have failed. M. I. Smith, however, found that tuberculous rats die with tuberculin shock when very large amounts of tuberculoprotein are injected into the blood stream. To explain the difference between the reported observations of others and those described in the present paper, it is noteworthy that the strain of tubercle bacilli used in our experiments is of unusually high virulence (0.00001 mg. injected intravenously kills a rabbit in from three to six months through extensive pulmonary and renal lesions), and the tuberculin employed is of high potency, approximately one and one-half times that of the international standard tuberculin.

The tuberculous rat is highly susceptible to the systemic action of tuberculin although its skin does not react to tuberculin. Cutaneous sensitization in the presence of systemic reactivity does not occur in young tuberculous guinea pigs (Freund;[22] Valtis [23]) and in tuberculous rabbits in the postallergic phase, i. e., when skin reactivity disappears preceding death (Freund, Laidlaw and Mansfield [24]). The systemic reaction to tuberculin appears to be specific even though tuberculous tissues are more susceptible to injurious agents than normal tissues (Bordet;[25] Freund [26]).

22. Freund, J.: J. Immunol. **13**:285, 1927; **17**:465, 1929.

23. Valtis, J.: Compt. rend. Soc. de biol. **99**:554, 1928.

24. Freund, J.; Laidlaw, E. H., and Mansfield, J. S.: J. Exper. Med. **64**:573, 1936.

25. Bordet, P.: Compt. rend. Soc. de biol. **106**:1251, 1931.

26. Freund, J.: Proc. Soc. Exper. Biol. & Med. **30**:535, 1933; J. Exper. Med. **60**:661 and 669, 1934; J. Immunol. **30**:241, 1935.

The only report in the literature on the formation of antibodies against tubercle bacilli in the tuberculous rat is that by Ornstein and Steinbach.[6] These authors found that complement-fixing antibodies were not produced in rats infected with a human strain of very low virulence (H37). We did not observe formation of antibodies in rats infected with virulent bovine tubercle bacilli. Nevertheless, complement-fixing antibodies were demonstrable in most of the infected rats that received one or more injections of tuberculin. Calmette and his co-workers [27] stated long ago that the injection of tuberculin promotes the formation of complement-fixing antibodies in tuberculous human beings.

The formation of antibodies was demonstrable in most of the rats that received repeated injections of heat-killed tubercle bacilli. Antibodies were produced in all of 4 animals that received intraperitoneal in addition to subcutaneous injections of heat-killed tubercle bacilli, and the antibody titer varied from 1 in 40 to 1 in 80.

Our observations show that rats are highly resistant to tuberculosis, for a large dose of highly virulent tubercle bacilli has not been fatal to them although tuberculous lesions, in most instances containing demonstrable tubercle bacilli, have been found in them after death. In these rats tuberculosis has progressed for only a short time and tubercles have not increased in size or number and have failed to caseate. The Royal Commission on Tuberculosis [13] and Ornstein and Steinbach [6] stated that tubercle bacilli multiply freely in tissues of the rat without inducing the formation of tubercles. We have found that the tubercle bacilli induce the formation of tubercles and that the micro-organisms are in epithelioid and giant cells. The number of micro-organisms in these cells is moderate in most of the rats, but tubercle bacilli in immense numbers were seen in the lungs of 4 rats that died one, six, twelve and eighteen months after infection. In most instances, though the disease has failed to progress, tubercle bacilli have persisted, and in a few animals they have undergone active multiplication and are found in great numbers within epithelioid and giant cells.

In the so-called rat leprosy, J. Jadassohn [28] and Muir [29] found enormous numbers of acid-fast bacilli in mononuclear cells. The micro-organisms were so numerous and closely packed that they appeared in

27. Calmette, A.; Massol, L., and Mezie, A.: Compt. rend. Soc. de biol. **73**: 122, 1912.

28. Jadassohn, J., in Kolle, W., and Wassermann, A.: Handbuch der pathogenen Mikroorganismen, ed. 3, Jena, Gustav Fischer, 1928, vol. 5, p. 1063.

29. Muir, E.: Leprosy, in A System of Bacteriology, Privy Council, Medical Research Council, London, His Majesty's Stationery Office, 1930, vol. 5, p. 379.

stained sections as red masses filling the cytoplasm. They were also present in giant cells and free in small blood vessels.

It is noteworthy that caseation of tubercles occurs in susceptible animals, such as the guinea pig and the rabbit, that acquire cutaneous sensitization to tuberculin, and is absent in the rat, in which the skin reaction does not occur. Nevertheless, caseation occurs in the tuberculous lesions of the lower monkeys though their skin is said to be refractory to tuberculin.

SUMMARY

Rats infected with highly virulent bovine tubercle bacilli fail to react with allergic inflammation to tuberculin injected into the skin but are killed through tuberculin shock by a small quantity of tuberculin.

Rats given repeated injections of heat-killed tubercle bacilli do not show cutaneous sensitization.

Rats immunized with heat-killed tubercle bacilli and subsequently infected are more susceptible to the systemic action of tuberculin than rats that have not been immunized.

Complement-fixing antibodies are not demonstrable in tuberculous rats, but they appear in infected animals after repeated injections of tuberculin.

Rats that receive several injections of heat-killed tubercle bacilli intracutaneously or subcutaneously show development of antibodies regularly; antibody formation is abundant after intraperitoneal injections.

After intravenous injection of 1 mg. of virulent bovine tubercle bacilli into the rat macroscopic tubercles develop in the lungs, the spleen becomes conspicuously enlarged, and microscopic tubercles are found in the spleen, in the liver and rarely in the kidneys. These lesions after approximately one month do not progress but persist until death. Tubercle bacilli are usually found in moderate numbers in epithelioid and giant cells. In a few instances tubercle bacilli are demonstrable in great numbers in histologic sections and are present within epithelioid and giant cells.

Tuberculosis in rats that have received repeated injections of heat-killed tubercle bacilli is similar to tuberculosis in previously normal animals, but in the former there is greater destruction of tubercle bacilli.

HISTOLOGY OF THE CUTANEOUS REACTION TO BRUCELLA MELITENSIS ANTIGEN

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In a recent study of the incidence of positive immunologic reactions for undulant fever, agglutination tests were done on 5,000 unselected blood samples, and skin tests were carried out as a routine on 491 of the patients whose bloods were tested.[1]

The agglutination and skin tests for undulant fever are extensively used as diagnostic aids. A positive skin reaction is generally believed to indicate bacterial sensitization resulting from past or present infection with Brucella.

A review of the literature on undulant fever failed to reveal reports dealing with the histologic picture of the skin reactions in man. For this reason, another series of intradermal tests was carried out, and a specimen of skin for biopsy was removed from each of the persons whose reaction was positive. Tissues were obtained at intervals from forty-eight to one hundred and ninety-two hours after the injection.

PROCEDURE

The antigen used for the intradermal tests was made from a strain of Brucella melitensis var. abortus, isolated from a patient with acute undulant fever. The strain was grown on a 2 per cent nutrient agar medium for forty-eight hours, suspended in physiologic solution of sodium chloride containing 0.5 per cent phenol, then diluted to a density of 30 (silica standard), which is about one-tenth the density of the usual brucella stock vaccine. The antigen was proved to be sterile.

For the test 0.1 cc. of this antigen was injected intradermally into the anterior surface of the forearm, and the reaction was observed forty-eight hours later. A positive reaction at that time showed a tender edematous area at the site of injection, with a central indurated area varying from 1 to 2 cm. in diameter and a peripheral zone of erythema from 2 to 8 cm. in diameter. An elliptic segment of skin measuring approximately 1 by 2 cm. was excised for histologic study from every patient who gave a positive reaction. The skin was removed after 2 per cent procaine solution had been injected into the normal skin around the zone of reaction. The tissue was excised by sharp dissection, and the edges

From the Department of Clinical Pathology and the Department of Pathology, University of Colorado School of Medicine and Hospitals.

1. Gersh, I., and Mugrage, E. R.: J. Lab. & Clin. Med. **23**:918, 1938.

of the skin were approximated with dermal suture. Prompt healing by first intention resulted. Cultures of the removed tissue proved sterile.

Specimens of skin were obtained from 12 patients: from 4 at forty-eight hours after injection; from 2 at seventy-two hours, from 2 at ninety-six hours, and from 1 each at one hundred and twenty, one hundred and forty-four, one hundred and

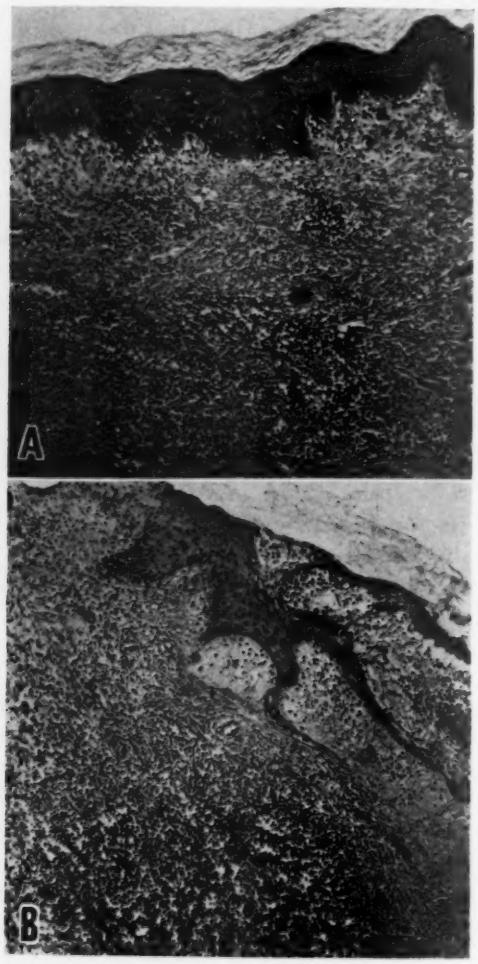

Fig. 1.—*A*, forty-eight hour reaction in the skin, showing infiltration of the derma by lymphocytes and monocytes. *B*, seventy-two hour reaction, showing edema and ulceration of the epidermis with extensive necrosis and leukocytic infiltration of the derma.

sixty-eight and one hundred and ninety-two hours. The specimens were fixed in solution of formaldehyde U. S. P. diluted 1 to 10 and were embedded in paraffin for sectioning. Sections were stained by Gram's method, with hematoxylin and eosin and with pyronin–methyl green.

HISTOLOGIC OBSERVATIONS

The following is a brief description of the changes noted in the histologic sections. The sections are described in groups according to the interval of time at which they were removed.

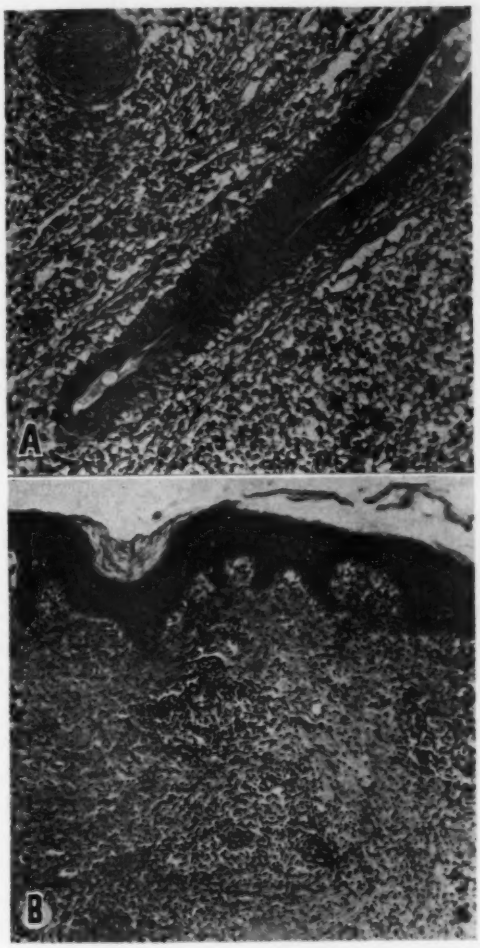

Fig. 2.—*A*, ninety-six hour reaction, showing perivascular infiltration. *B*, one hundred and twenty hour reaction, showing characteristic lymphocyte-monocyte infiltrate in the derma.

In the forty-eight hour specimens the epidermis showed no evident change in 1, from slight to moderate edema in 2 and marked edema with formation of blebs in 1. There was from slight to marked edema of the derma with a cellular infiltrate composed of lymphocytes and mono-

cytes or histiocytes in all 4 (fig. 1 *A*). There was focal necrosis of connective tissue in 2, and in these polymorphonuclear leukocytes were numerous, especially in the areas of necrosis. Gram-negative coccoid bacilli resembling Br. abortus, located extracellularly, were present in 1 but were not demonstrable in the other 3. Identification of Br. abortus in the remaining sections of the other groups proved to be impossible. In a specimen in which focal necrosis was present several of the larger blood vessels had undergone thrombosis.

One of the seventy-two hour specimens showed the epidermis normal. In the other the epidermis was edematous and showed a central shallow ulcer. In the first specimen there was slight and in the second marked edema of the derma. Both specimens showed dense leukocytic infiltration, which was predominantly lymphoid in the first, while in the second there was a large admixture of polymorphonuclear leukocytes accompanying extensive necrosis of connective tissue (fig. 1 *B*).

One of the ninety-six hour specimens showed normal epidermis except at the site of the needle puncture, where the break in continuity was covered by a thin crust of desiccated fibrin. One disclosed slight edema of the derma; the other, none. Both showed the characteristic lymphocyte-monocyte infiltrate, with polymorphonuclear leukocytes concentrated in areas of necrosis (fig. 2 *A*).

In the specimen obtained at one hundred and twenty hours the epidermis was normal and the derma free from edema. There was an infiltrate of leukocytes, chiefly lymphocytes, but with many monocytes present, some of which were in mitosis. A small zone of necrosis containing polymorphonuclear leukocytes was present (fig. 2 *B*).

In the specimens taken at one hundred and forty-four, one hundred and sixty-eight and one hundred and ninety-two hours edema was slight or absent. The infiltrate was dense and tended to be diffuse. The infiltrating cells were of the same types as in the earlier specimens, but the monocytes were more numerous with occasional nuclei in mitosis. Evidence of phagocytosis of lymphocytes by these monocytes was seen. Large necrotic zones, in which the connective tissue fibers were fragmented, were densely infiltrated by polymorphonuclear leukocytes, and in some instances abscesses had formed. Such zones were bordered by the usual lymphocyte-monocyte infiltrate, with polymorphonuclear leukocytes becoming less numerous toward the periphery.

COMMENT

Certain general features of the reaction in the skin as seen microscopically are common to all the specimens examined. The reaction consists of a cellular infiltrative process, in which there is slight to

moderate edema with collections of cells in the loose areolar connective tissue of the corium around blood vessels, nerves, hair follicles and sweat and sebaceous glands. Where the infiltrate extends into the panniculus adiposus it follows irregularly branching pathways of loose perivascular connective tissue.

The epidermis and the coarse collagenous connective tissue fibers of the derma are not altered in the milder reactions, but in the more severe ones necrosis occurs. Small foci of necrosis are present in the derma as early as forty-eight hours. The epidermis is not the primary site of necrosis but may undergo secondary changes with bleb formation and ulceration.

The tissue reaction following intradermal injection of Br. abortus vaccine may be assumed to vary in intensity with the degree of sensitivity of the tissues and with the amount and concentration of vaccine injected. As the strength of the vaccine used was constant, the difference in severity of the tissue reactions in different persons with the same interval of time is thought to be dependent on the difference in their sensitivity to the brucellas. Apart from the individual difference in sensitivity, the older reaction exhibits more extensive destruction of tissue, with little evidence of resolution, indicating that the reaction is progressive and reaches its height after forty-eight hours or more. In this and in other respects it resembles the reaction to tuberculin as described by Dienes and Mallory.[2] It differs from the latter reaction, however, in that the necrosis begins in the corium rather than in the epithelium.

The infiltrating cells are predominantly lymphoid, with variable numbers of large mononuclear cells of the histiocyte or monocyte type. Plasma cells are present but not numerous. Giant cell formation is not observed. Hyperemia is not prominent in any of the specimens. In the absence of necrosis polymorphonuclear neutrophilic leukocytes are few and scattered, but where necrosis is present large numbers of these cells occur, collecting in and about the necrotic zone. Eosinophilic leukocytes are not found. The observations of Dienes and Mallory[2] as regards the tuberculin reaction suggest that the polymorphonuclear reaction is secondary to necrosis of epithelium. The association of these leukocytes with necrosis and their relative scarcity in the tissues showing milder changes due to Br. abortus infection had been noted by Hallman, Sholl and Delez.[3]

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2. Dienes, L., and Mallory, T. B.: Am. J. Path. **8**:689, 1932.

3. Hallman, E. T.; Sholl, L. B., and Delez, A. L.: Observations on the Pathology of Bacterium Abortus Infections, Technical Bulletin no. 93, Michigan State College, Agricultural Experiment Station, 1928.

SUMMARY

The tissue reaction to the intradermal injection of Br. melitensis var. abortus antigen in 12 persons giving a positive reaction has been studied histologically. The reaction is inflammatory. When it is mild, it is characterized by infiltration of the derma by lymphocytes and monocytes; when it is severe, it is accompanied by connective tissue necrosis and infiltration by polymorphonuclear leukocytes.

Case Reports

BOTRYOMYCOSIS

Report of Two Cases of Intra-Abdominal Granuloma

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We have recently observed 2 cases of granuloma of the abdominal cavity in which botryomycotic granules could be demonstrated.

CASE 1

A colored man aged 33 years was admitted to the hospital division of the Medical College of Virginia three times during 1936, the first time on July 6.

He stated that for three weeks he had had severe cramplike pain just above the umbilicus. The pain radiated to and fro across the abdomen and was worse immediately after eating. Drinking cold liquids caused paroxysms of pain. He noticed a tender mass in the midupper part of the abdomen. The stools became more frequent and were loose and watery. Ten years previously he had received antisyphilitic treatment. He was well developed. There was marked dental caries. The abdomen showed a mass 2 inches (5 cm.) in diameter midway between the xiphoid process and the umbilicus. This disappeared when the abdominal muscles were contracted. It was slightly tender; it was fixed; no sounds were heard over it on auscultation, but it transmitted the aortic pulsations. There was generalized lymphadenopathy.

Roentgen examination revealed a filling defect in the middle portion of the transverse colon, apparently due to an extrinsic mass. A barium sulfate enema showed a sharply defined irregularity of the inferior border of the transverse colon for 2 inches in the midportion.

Urinalysis gave negative results. The red blood cell count was 3,920,000. The hemoglobin content was 67 per cent (Sahli). The white blood cell count was 8,100, with polymorphonuclears 58 per cent and lymphocytes 42 per cent. The Wassermann and Klein reactions of the blood were positive.

Exploratory laparotomy (Dr. H. J. Warthen) in July 1936 revealed an inflammatory mass 5 cm. in diameter arising from the right side of the transverse colon and densely adherent to the round ligament, omentum and jejunum. No obstruction was noted. The stomach and retroperitoneal tissues were free. An appendectomy was done. The abdomen was closed in layers, without drainage. Cultures from the mass showed colon bacilli and Staphylococcus albus.

The postoperative course was uneventful. A barium sulfate enema August 3 showed considerable diminution of the obstruction of the transverse colon. The patient was discharged August 5.

He was readmitted August 12 with abdominal pain suggesting a partial intestinal obstruction. He improved under conservative treatment. A barium sulfate enema showed slight irregularity of the transverse colon. He was discharged August 19 in good condition.

From the Department of Pathology, Medical College of Virginia.

He was admitted again August 28, stating that he had noticed a tender swelling in the lower part of his incision for twenty-four hours. There was a rather firm nonfluctuant tender mass in the lower portion of the old incision, to the right and just above the umbilicus. Two days later an abscess in the abdominal wall was emptied, 2 ounces (59 cc.) of pus being obtained. A culture revealed Staphylococcus.

An acute mechanical intestinal obstruction developed. September 18, a laparotomy (Dr. H. J. Warthen) showed the transverse colon to be bound into a dense inflammatory mass with three loops of small intestine. There was marked constriction of these loops. The mass was excised with V-shaped areas of the small intestine and colon. A colostomy through a McBurney incision on the right side was done. The wound was closed in layers, with drainage. A fish bone 1 inch (2.5 cm.) long was found in the midst of the mass.

Massive atelectasis of the right lung developed. A bronchoscopic aspiration was done September 22 for the atelectasis, with little improvement. The temperature curve was of a septic type. The patient received two transfusions and supportive treatment. The wound became grossly infected. The condition became progressively worse, and the patient died October 12. Permission for a postmortem examination was refused.

The mass removed at the last operation, measuring 7 by 3.5 by 2.5 cm., was attached to a piece of large intestine. It was firm in consistency, and its cut surface was light gray and homogeneous. The center of the mass contained a few well defined soft areas, which were rather dark red with ochre yellow centers (fig. *A*, left).

Histologically, the mass consisted of a dense acellular connective tissue with scanty vessels and in several places hyaline degeneration. A few scattered areas of perivascular round cell infiltration were noted. Within the areas there was a rather cellular granulation tissue composed of fibroblasts and numerous large mononuclear foam cells, numerous foreign body giant cells and an irregular infiltrate of lymphocytes and plasma cells. Russell's bodies were frequently encountered. Polymorphonuclear cells, however, were almost absent. The areas were rather vascular. Although there was no evidence of recent hemorrhage, the presence of numerous iron-pigmented macrophages indicated old hemorrhage. The tissue contained several small, well circumscribed abscesses with complete liquefaction of tissue and dense infiltration with polymorphonuclear cells. In the centers of some of these abscesses, granules were found (fig. *B*). Under low power magnification they resembled the "sulfur granules" in actinomycosis. With the hematoxylin and eosin stain they were rather dark blue and irregularly finely granular, within dense dark bluish borders. These granules were surrounded by a narrow pinkish-stained hazy zone, which in some places was more condensed and formed clublike excrescences. A doubly refractive membrane was absent. The Gram stain showed that the granules contained numerous gram-positive cocci, which were concentrated at the borders, although they were not found within the clublike excrescences themselves. The cocci were mostly arranged in rather long chains resembling streptococci. In some places they were clumped together. No cocci were demonstrable outside the granules. The matrix of the granules did not take the Gram or a fibrin stain. The centers of most of these granules stained reddish with the Kernechtrot [1] counterstain.

1. The formula of the Kernechtrot counterstain is as follows: One-tenth gram of Kernechtrot (obtainable from Pfaltz and Bauer, New York) is dissolved in 100 cc. of 5 per cent solution of aluminum sulfate by boiling. The sections are stained for from two to five minutes and washed in water without further differentiation.

CASE 2

A white woman 51 years of age was admitted to the hospital division of the Medical College of Virginia, to the surgical service, Oct. 19, 1936, because of a mass in the right lower quadrant of the abdomen, which had been present for five or six months. At times this mass caused considerable pain. The woman had complained of chronic constipation over a period of years. She had no diarrhea, no blood in the stools and no digestive disturbances. She was totally deaf. Her physical condition was good except for a firm rounded mass in the right lower quadrant of the abdomen, apparently fixed to the inner surface of Poupart's ligament. It was approximately 3 cm. in diameter. Roentgenograms of the pelvis showed nothing of importance except slight hypertrophic arthritis of both hip joints.

Urinalysis gave negative results. The red blood cell count was 3,900,000. The hemoglobin content was 78 per cent (Sahli). The differential count showed 72 per cent polymorphonuclear leukocytes and 28 per cent lymphocytes. The Klein and Wassermann reactions of the blood were positive.

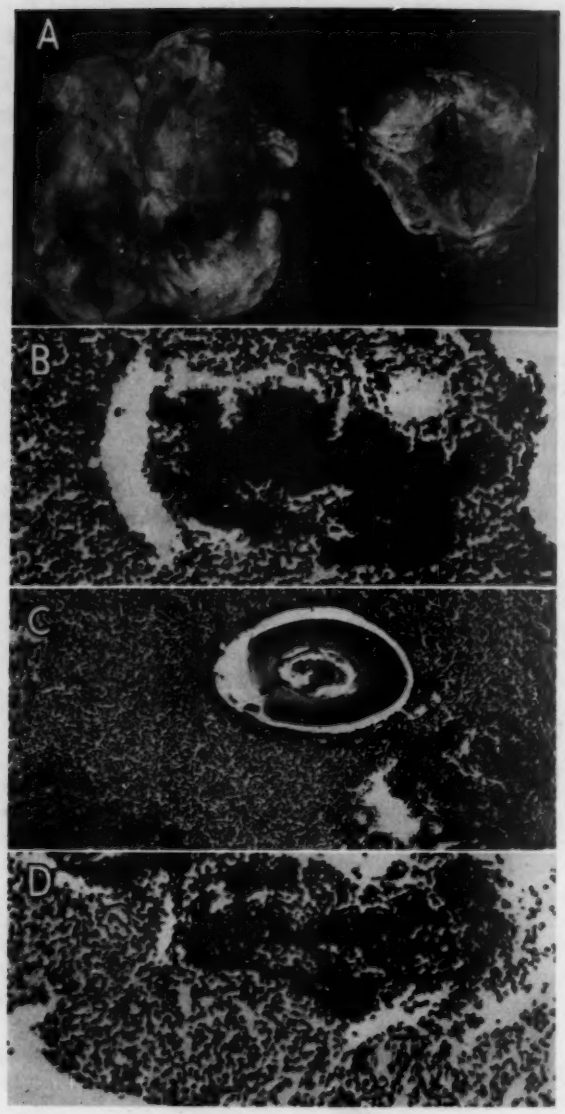
At operation a low incision of the right rectus muscle was made, and the omentum was found adherent to the parietal peritoneum. A mass was present involving the peritoneum and the internal and external fascial sheaths of the rectus muscles. The entire mass was excised within a block of the abdominal wall. A fish bone was found in the mass. The abdomen was closed in layers, without drainage. The patient made an uneventful recovery and was discharged completely healed on November 4.

The gross specimen measured 5.5 by 2.5 by 2 cm. On the cut surface it was homogeneously gray except for a few small ill defined reddish-colored areas (fig. *A*, right). The histologic appearance was that of a dense fibrous tissue with an irregular infiltrate of small round cells. In the majority of blocks the tissue was acellular. In some of them, however, were areas of a cellular granulation tissue. Foreign body giant cells were scattered throughout the entire mass. In one of the areas a fragment of a fish bone was found, which was impregnated with iron salts (positive Perl reaction). There were no polymorphonuclear cells around the fish bone (fig. *C*). In its vicinity were several small foci in which there was a peculiar degeneration with the nuclei of the fibrous connective tissue densely crowded together, greatly swollen and vacuolated, so that they presented a honeycomb appearance. The paraplastic substance apparently had undergone liquefactive necrosis, and the entire focus under low power magnification was irregularly dark bluish. There was no polymorphonuclear cell reaction within or in the vicinity of such foci.

In two places, however, the bluish-stained mass of necrotic tissue was condensed and formed clearly delineated granule-like bodies stained dark blue with hematoxylin and eosin and separated from the surrounding tissue by a rather narrow zone of polymorphonuclear cells (fig. *D*). These granules were not as sharply demarcated as those in case 1; their borders were much more heavily stained than their centers. No clublike excrescences and no membrane could be demonstrated. The Gram stain revealed that the entire mass was free from cocci except the areas of necrobiosis described. Staphylococci were scattered within these foci. The granules themselves, which were surrounded by polymorphonuclear cells, revealed a few staphylococci peripherally.

PATHOGENESIS OF BOTRYOMYCOSIS

This condition, although frequently encountered in the practice of veterinary medicine, as castration fungus in horses, is rarely mentioned as having been observed in man. Six cases of the disease in man seem



A, gross appearance of the two inflammatory tumors (right, case 1; left, case 2). *B*, granule within an area of suppuration in case 1; hematoxylin and eosin stain. Delicate raylike excrescences can be recognized at the border. *C*, fish bone in case 2. The dark stained area is composed of densely crowded swollen nuclei without polymorphonuclear cell infiltration. *D*, condensing mass of débris within an area of suppuration—beginning formation of a granule. Bacteria are found only within this area (case 2).

to be known. Reference to 5 of them, including their own, is found in the paper of Berger and his co-workers.[1a] The sixth case was reported by Plaut.[2]

Botryomycosis is a chronic inflammatory lesion in which peculiar granules are found, the nature of which was first determined by Magrou.[3] Under low power magnification these bodies resemble the sulfur granules in actinomycosis because of their clublike excrescences. They are situated in small foci of suppuration. The granule itself consists of an unidentified matrix, probably a bacterial and tissue débris, in which bacteria can be demonstrated. The latter are usually crowded in the periphery, and the granule in its characteristic form is surrounded by a doubly refractive membrane. Magrou showed that the lesion is caused by staphylococci.

In all cases except Berger's it has been reported that foreign bodies participated in the development of botryomycosis. In the castration fungus of horses, hairs are found. Bony sequestrums and fish bones are present in the lesions in man. It is, however, still in question whether and how much the foreign body itself contributes to the specific character of the condition. Magrou stated that the lesion can be reproduced in animals in the absence of foreign bodies by inoculating staphylococci, provided the quantity is small enough not to give rise to suppuration and large enough to initiate a slow development of granulation tissue. To our knowledge, his experiments have not yet been reproduced. The fact, however, can hardly be neglected that in 7 (including both our cases) of 8 instances in men foreign bodies were present.

It is furthermore questionable whether in cases of spontaneous botryomycosis in man the limited number of bacteria should be regarded as the main factor in the development of the lesion. Their invasion and multiplication depend largely on the tissue reaction, which therefore must be taken into consideration. Berger and his associates discussed the possibility that it is the type of tissue which inclines to such a reaction. This, however, appears to be unlikely since botryomycosis is found in the scrotal tissue of horses and in the peritoneal, bony and genital tissues of human beings. These authors suggest further that colon bacilli, which in their cases were found together with staphylococci, may have secreted antiphagocytotic substances thus controlling tissue necrosis and reaction. This explanation, however, does not apply to instances in which staphylococci only are found.

The type of bacteria or their virulence does not seem to be important. Magrou was able to obtain identical results in his experiments with ordinary staphylococci as well as with those isolated from spontaneous botryomycosis. Colon bacilli were found to accompany the staphylococci in the case observed by Berger and in our case 1.

ANALYSIS OF OUR CASES

In our first case the bacteria were arranged in scanty, scattered granules which more or less had the characteristics of what is called

1a. Berger, L.; Vallee, A., and Vezina, C.: Arch. Path. **21**:273, 1936.

2. Plaut, A.: Arch. Path. **23**:602, 1937.

3. Magrou, J. E.: Les grains botryomycotiques: Leur signification en pathologie et en biologie générales, Thesis, no. 267, Paris, Laval, 1914.

botryomycosis. It is questionable, however, whether the second case can be identified as one of botryomycosis. In fact, our histologic observations are not compatible with the present concept of this condition. The granules were not surrounded by a cuticula nor were they garnished with clubs. They did, however, bear a great resemblance to those of botryomycosis in that they consisted of a rather homogeneous mass of débris which contained cocci. The borders were deeply stained with hematoxylin and sharply delineated, and the granules were embedded in discrete foci of suppuration. The identity of both cases, furthermore, is based on the fact that the lesions were similar in their clinical development and were grossly alike, both containing a fish bone. In both cases, staphylococci were found in discrete areas of débris.

We assume that in our second case an early stage of the formation of granules was demonstrated. The first change we observed was that of degeneration of rather dense connective tissue in discrete areas. This degeneration was probably due to action of bacteria, which were found exclusively within such foci. The intercellular material appeared to liquefy, and the nuclei of the fibroblasts underwent marked vacuolar disintegration and swelling. They crowded together, and the chromatin material diffused through the nuclear membrane, which finally disintegrated. The irregularly bluish-stained focus was at first ill defined but with increasing condensation became more sharply bordered. The bacteria remained alive within this shrinking ball of débris, and there was remarkably little cellular reaction until the granule was fairly well formed. It was only at this period of development that a polymorphonuclear cell exudate commenced to surround the finally completely detached granule, which then appeared to float in pus.

COMMENT

Although the sequence of events can be demonstrated well in one of our cases, there was nothing that explained clearly why the multiplication of bacteria in botryomycosis is limited to growth within granules. Several factors may be considered: In the first place, the granule may be surrounded by a cuticula. Masson [4] explained the process in the presence of bone spicules as follows: The staphylococci multiply in the shelter of haversian channels. As soon as the bony substance is destroyed and the clusters of bacteria come in contact with the surrounding tissue, a membrane precipitates.

In the absence of bone protection, however, a different mechanism has to be assumed. Berger and his co-workers mentioned the possibility that the cuticula may result from coagulation necrosis of connective tissue fibers and that staphylococci multiply within its shelter, thickening the membrane by internal apposition of protein derived from dead cocci. Since the membrane itself is described as hard and breakable under the knife, it is unlikely that further enlargement of granules could occur. Thus, the growth of granules is terminated by the precipitation of such a membrane. In the early phases of development, however, the membrane must be of a more plastic nature, and Berger and his associates described it as fibrin-like, although it does not show specific staining properties. They interpreted such membranes as "probably beginning

4. Masson, P.: Lyon chir. **15**:230, 1918.

shells." It therefore appears to us that the characteristic doubly refractive hard membrane rather represents a terminal phase in the development of the granules and that little is known about the time of its appearance or of its nature in the early stages.

Not all such granules, however, are surrounded by a distinctly visible doubly refractive membrane. Plaut stated that in his case the periphery of each granule was formed by a homogeneous mass obviously consisting of coalescing dead cocci. The observations in our first case are identical with his, but the granules in our second case did not reveal a membrane; the growth of bacteria had been limited to discrete areas without the formation of a mechanical visible barrier.

An adequate explanation of this phenomenon cannot be given yet. It does not appear to be determined by the type of bacteria only, since staphylococci and staphylococci mixed with colon bacilli have been found, nor is the bacterial growth limited by the precipitation of a membrane, since the latter may be absent. We are of the opinion that more emphasis should be laid on the tissue reaction itself, since in the majority of cases foreign bodies have played a role. This suggests that tissue thus irritated differs in its defense mechanism from virginal tissue.

Finally, it should be mentioned that the clublike excrescences may be absent as in the spontaneous lesions of horses. They may vary in number, some of the granules being bare, others provided with clubs. Hence we conclude that both the doubly refractive membrane and rays or club formations are of an accessory nature.

SUMMARY

Two cases of intra-abdominal granuloma are presented, in both of which the granuloma contained botryomycotic granules. The early phases of the development of the granules are described. It is emphasized that foreign bodies play an important rôle in the pathogenesis of this lesion.

MEDIAL DEGENERATION IN A NONRUPTURED AORTA APPEARING SYPHILITIC MACROSCOPICALLY

ANTONIO ROTTINO, M.D., NEW YORK

Medial degeneration has to date been described largely in cases of spontaneous rupture of the aorta. This paper records an instance of severe medial degeneration in which the aorta dilated but did not rupture. It will serve, further, to emphasize that in some instances gross changes may occur which to the naked eye appear like those of syphilis. This real pitfall was alluded to by Erdheim,[1a] who stated that for some time in demonstrating the lesions of syphilis he had used material which to his amazement the microscope later showed to be nonsyphilitic. This occasioned two reports in which he described a new disease, "medio-necrosis idiopathica cystica."[1b]

REPORT OF A CASE

A 70 year old woman entered St. Vincent's Hospital, in the medical service of Dr. Thomas A. Martin, Sept. 19, 1936, with congestive heart failure. Her symptoms appeared six months before and increased in severity until she was edematous, dyspneic, and orthopneic at rest. After two months' rest in bed and treatment with digitalis she recovered sufficiently to get up. In three weeks, however, she was again seized with congestive heart failure and therefore entered the hospital.

She was dyspneic and orthopneic, with cyanosis of the mucous membranes and pronounced edema of the lower extremities. The pupils were equal and active. The veins of the neck were dilated. Moist rales were heard at the bases of the lungs. Percussion showed the heart to be enlarged. The sounds were poor; no murmurs were heard. The rhythm was irregular and the rate rapid, 158 beats per minute. The blood pressure was 134 systolic and 78 diastolic. The Kahn test of the blood was negative. The blood sugar was 111 mg. per hundred cubic centimeters. The urine contained albumin (1 plus). An electrocardiogram revealed auricular flutter with varying degrees of block and occasional premature ventricular contractions.

After five days of increasing failure of the heart, the patient died.

Necropsy.—The examination was made twenty-four hours post mortem.

The heart lay free in the pericardial cavity, was generally enlarged and weighed 415 Gm. All the chambers were moderately dilated, while the ventricles were in addition hypertrophied. No unusual valvular changes were noted. Beneath the aortic valve several endocardial pockets were found on the interventricular septum despite normal cusps and commissures. The coronary ostia were wide and fully patent, while the arteries leading from them were thick, tortuous and calcified but unobstructed.

From St. Vincent's Hospital.

1. Erdheim, J.: (*a*) Virchows Arch. f. path. Anat. **273**:454, 1929; (*b*) **276**: 187, 1930.

The ascending aorta was transformed into a diffusely dilated, nonelastic, thin-walled sac (12.5 cm. in circumference) which ended abruptly in a moderately dilated arch. The descending aorta was likewise moderately dilated. The intima lining the aneurysm was thick, gray and wrinkled, containing numerous pearly plaques, giving it the appearance of tree bark so often seen in syphilis. The usual yellow of the underlying media was either entirely absent or obscured by the opaqueness of the thickened intima. Beyond the aneurysm the intima for the most part was thin and transparent, so that the underlying, grossly intact yellow media was easily observed. Atheromatous plaques were scattered about the arch as well as around the mouths of the large branches arising from the abdominal aorta. Some were calcified and others ulcerated. The lower portion of the thoracic aorta and the abdominal aorta were entirely smooth.

Other findings were as follows: There was a moderate amount of edema of the legs, with 200 cc. of fluid in the abdominal cavity and 1,000 cc. in the pleural sac, the latter causing some compression atelectasis of the lungs. Two small infarcts were observed in the lower lobe of the right lung. The liver, though not enlarged, presented the nutmeg markings characteristic of chronic passive congestion. The ovaries were sclerotic and the uterus atrophic. The kidneys together weighed 315 Gm. Their surfaces were finely granular. In one kidney two small infarcts were found.

Anatomic Diagnosis.—The diganosis was: aneurysm of the ascending aorta, probably syphilitic (this was the diagnosis made at the autopsy table; later, when the slides were seen, the diagnosis was changed to medionecrosis idiopathica); calcification and sclerosis of the coronary arteries; bilateral pleural effusion; infarcts of the lower lobe of the right lung; compression atelectasis of the lower lobes of both lungs; chronic passive congestion of the liver and spleen; arteriolonephrosclerosis; infarct of the kidney, and senile atrophy of the uterus and ovaries.

Microscopic Observations on Aorta.[2]—In general, the intima appeared as a wavy, variably thickened layer, here thin, there thick. The thickened portions were in the main composed of dense hyalinized fibrous tissue, in which were intermingled varying amounts of fine elastic fibrils and collagen. In some areas large quantities of calcium were deposited, while here and there were scattered a few muscle cells.

In addition to exhibiting diffuse thickening, the intima contained raised plaques. Most of them consisted of dense fibrous tissue overlying lipoid cavities. Others were entirely fibrous, calcified and even ossified. Beyond the aneurysm the intimal changes were not pronounced.

The continuity of the internal elastic lamina in the ascending aorta was frequently interrupted. In some regions it stood out thick and deeply stained, while in other places it grew pale and disappeared. In some areas where it was absent it became difficult to decide where the thickened intima ended and the media began.

2. The entire length of the ascending aorta was sectioned into serial blocks. The sections extended from just below the aortic valve ring to the beginning of the arch. In addition, shorter transverse blocks were cut from the arch, thoracic aorta and abdominal aorta. They were fixed in solution of formaldehyde U. S. P. and embedded in paraffin, and sections were stained with hematoxylin and eosin, Weigert's stain for elastic tissue, Van Gieson's stain for connective tissue, Mallory's phosphotungstic acid-hematoxylin stain and the Foot and Foot stain for reticulum (Am. J. Path. **8**:245, 1932).

The media presented the principal changes, for it had become markedly altered. Histologically, the lesions varied considerably. They were most conspicuous toward the center of the aneurysm, where the media was distinctly thinner than normal, being sandwiched in between the thickened intima and adventitia. The

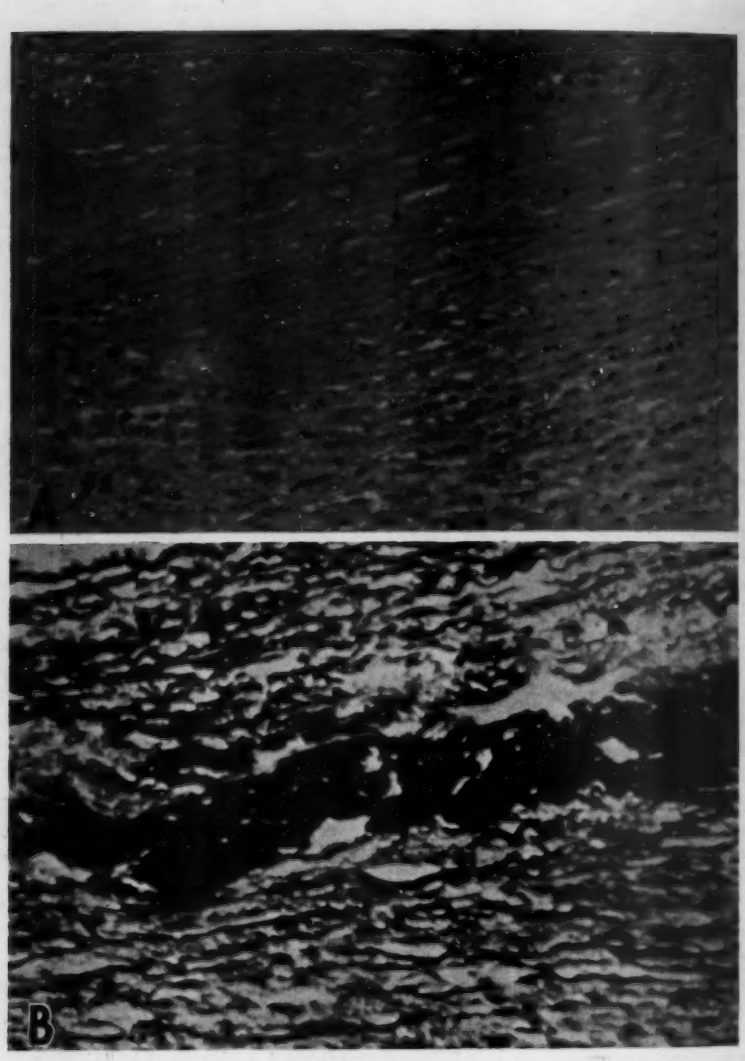


Fig. 1.—*A*, low power magnification of an area of medionecrosis (Gsell[3]); hematoxylin and eosin. Note focal loss of muscle cells. A few pyknotic nuclei remain. The surrounding muscle cells appear normal. *B*, low power magnification of an area of medionecrosis; Weigert's stain for elastic tissue. Though muscle cells are gone, the elastic elements remain, densely stained, thick, compressed, obliterating the interlamellar spaces.

changes were less pronounced at the aortic root and arch, which corresponded to the periphery of the aneurysm. They were absent in the descending portion of the aorta.

One type of lesion was particularly conspicuous (fig. 1 *A*), as many as twenty-seven lesions of this type being counted over a strip 7 cm. long. They were

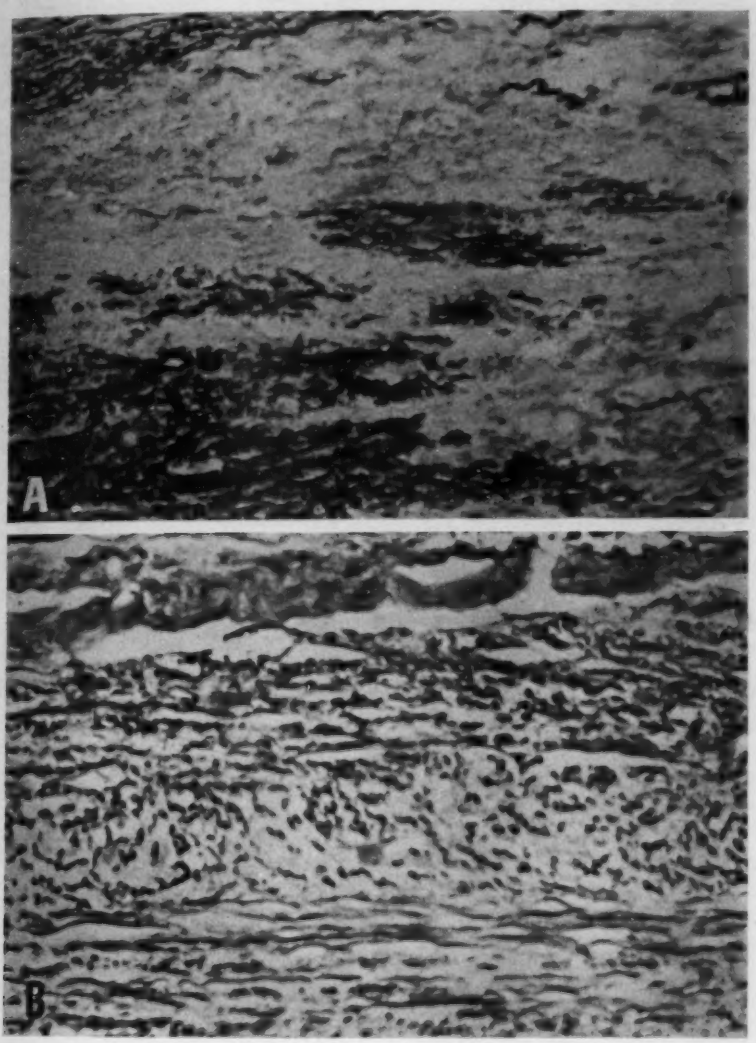


Fig. 2.—*A*, low power magnification of irregular areas devoid of elastic laminae; Weigert's stain for elastic tissue. At the margin the elastic laminae end abruptly. *B*, low power magnification of the contents of the defects illustrated in *A*; hematoxylin and eosin. Numerous muscle cells are seen, compactly and irregularly arranged. Sometimes elastic and collagen fibrils are also present.

distributed from one end of the aneurysm to the other, in one direction, and about the entire circumference, in the other. The portion of the media involved varied. Most affected was the midportion, next the outer third and least affected was the inner third. The lesions varied in size, some occupying a small portion

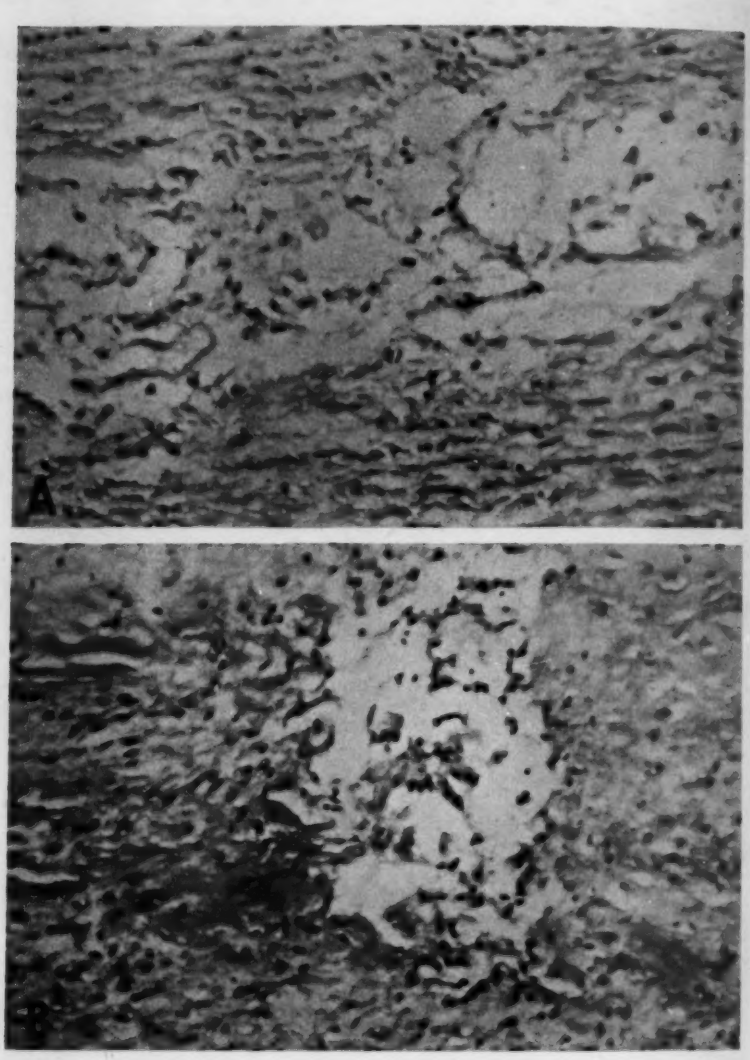


Fig. 3.—*A*, low power magnification of a medial defect from which the elastic tissue is gone but which contains a few muscle cells widely dispersed by mucoid material; hematoxylin and eosin. *B*, low power magnification of a mucoid cyst containing muscle cells; hematoxylin and eosin.

of a low power field, while others measured 3 mm. in length. They involved as much as one-third to one-half the thickness of the media. With the hematoxylin and eosin stain they stood out as deeply eosinophilic areas, devoid of almost all

muscle cells. In a few of these areas nuclei were still present, either pale and swollen or small and pyknotic. With the elastic tissue stain (fig. 1*B*) the elastic lamellae were seen deeply stained, thick, straight and crowded together so that the interlamellar spaces were much narrowed and the collagen compressed, as a result of which the latter frequently stood out plainly when stained with Van Gieson's stain for connective tissue or Mallory's phosphotungstic acid–hematoxylin stain. The reticulum which normally hugs the lamellae persisted. In an occasional lesion the elastic laminae were fragmented and pale staining.

A second type of lesion observed (fig. 2*A*) was just as conspicuous, with approximately the same distribution. It was seen to best advantage in sections treated with Weigert's stain for elastic tissue, in which it appeared as irregular gaps at the margins of which the elastic laminae ended abruptly. In sections stained with hematoxylin and eosin (fig. 2*B*) it was recognized by the large number of muscle cells present, arranged in planes different from that of the normal cells in adjacent areas; i. e., their long axes pointed obliquely or perpendicularly to the plane of the normal cells. In addition, there were occasional areas in which scant amounts of collagen and elastic tissue fibrils intertwined about these cells. In some lesions (fig. 3*A*) the cells were less numerous and less compactly arranged, being separated by varying amounts of mucoid material. In one field the amount of the latter was considerable, forming a cystlike space in which were suspended remnants of old media (fig. 3*B*).

The adventitia in general was thick and composed of dense hyalinized connective tissue. The blood vessels were not increased in number and for the most part were small and unchanged. Only a few exhibited mild intimal thickening. Scattered throughout the adventitia were collections of lymphocytes. In one area only were any present in considerable numbers. They were chiefly located in the outermost portion of the adventitia, bearing no relationship to the vasa vasorum.

COMMENT

The pertinent finding in this case is the occurrence in a 76 year old woman of a marked aneurysmal dilatation of the ascending aorta, with wrinkling and puckering of the intimal surface identical with that seen in syphilis. Histologic study showed, however, that the lesion was instead one described by Gsell,[3] Erdheim[1] and others in cases of spontaneous rupture of the aorta.

The lesions as seen in the case reported here were focal and characterized by absence of one or more of the normal components of the media. In one lesion there was loss of muscle; in a second the muscle cells were unusually plentiful but the elastic lamellae had disintegrated. Then there were other areas in which abnormal amounts of mucoid material separated the medial elements. Inflammatory reaction and fibrous tissue repair were characteristically absent.

The pathogenesis of the disease is imperfectly understood. It is to Gsell that one owes some of the knowledge of this subject. He felt that the initial lesion was focal necrosis of muscle cells, subsequent to which the remaining components in the same area, without aid of cellular reaction, disintegrated. Following this the defect was repaired by a tissue composed of scant, loose collagenous fibers and a few elastic fibrils. A blood vessel might or might not grow into the area.

3. Gsell, O.: Virchows Arch. f. path. Anat. **270**:1, 1928.

Thus the process appeared to consist of three steps—first, necrosis; second, humoral dissolution, and last, repair by an imperfect type of scar. Erdheim amplified the theory by describing more completely the picture in the second stage—the stage of dissolution. He felt that he could demonstrate as a result of the latter process spaces or cysts filled by fluid which stained metachromatically with thionine and cresyl echt violet. To the stage of healing he further added an alternative to Gsell's "imperfect scar," namely, regeneration of muscle. In his opinion, the defects left in the wake of dissolved necrotic areas might become filled again by a regeneration of all the original components, particularly muscle, with this difference, that the normal medial pattern was not reproduced. Instead, the muscle cells were laid down in various positions, with the elastic tissue forming a loose web about them. Finally, he felt that in addition to primary necrosis of muscle the media could undergo destruction by increasing accumulations of mucoid between the lamellae. With confluency of these small collections of mucoid and concomitant disappearance of muscle and thinning of the elastica, mucoid cysts might form.

Much that was described by these two men was seen in the case reported here. Areas of loss of muscle cells were numerous. Unlike the observations in Gsell's cases, however, the anuclear necrotic cytoplasmic remnants of muscle cells were not evident. Also present were areas of the type interpreted by Erdheim as examples of regenerated media. Scar tissue repair as described by Gsell was absent.

As stated medionecrosis has been described chiefly in cases of spontaneous rupture of the aorta. Reference to its presence in intact vessels are few. Weise [4] studied 120 aortas obtained at necropsies and found medionecrosis in the sense of Gsell in 9 of them. In each the lesion was microscopic and did not lead to macroscopic alteration of the wall. Cellina [5] selected 10 aortas with a minimal amount of atherosclerosis from persons over 72. In 9 he found focal loss of muscle cells but of a type which he felt was different from that described by Gsell. To the lesion which he observed he appended the name "medionecrosis disseminata"; to Gsell's, "medionecrosis idiopathica." Moritz [6] alluded to the presence of macroscopic mucoid cysts in several intact aortas.

Another unusual observation in this case was that of intimal change. In reporting aortas which ruptured spontaneously and exhibited medionecrosis, all authors have made it a special point to emphasize the absence of intimal reaction. This lack may have been one of the factors responsible for the ruptures in those aortas and the presence of this reaction in the instance which I have described may explain the failure of the aorta to rupture. Adventitial thickening beneath areas of medionecrosis was described by Gsell. In the case now reported it was likewise present to a marked degree over the entire extent of the aneurysm.

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4. Weise, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:238, 1934.
5. Cellina, M.: Virchows Arch. f. path. Anat. **280**:65, 1931.
6. Moritz, A. R.: Am. J. Path. **8**:717, 1932.

The causes of the disease are entirely unknown. What theories have been advanced are discussed by Shennan.[7]

SUMMARY

A case of aneurysm of the aorta macroscopically mistaken for syphilis but microscopically analogous to the disease described as medionecrosis is presented. The aneurysm occurred in a 76 year old woman, who died in congestive heart failure.

The case is unique since in other recorded instances of advanced medionecrosis the aorta ruptured spontaneously whereas in this case it remained intact.

7. Shennan, T.: Dissecting Aneurysm, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

AN UNUSUAL FREE BODY IN THE TUNICA VAGINALIS TESTIS

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There has been no recent comprehensive review of the literature on the subject of the free body of the tunica vaginalis testis. Schmidt [1] in his paper on the broader subject of free bodies in serous cavities covered some of the older papers on the subject, and Ritter [2] gave a fairly extensive review of the theories that have been advanced as to the origin of these bodies. The opportunity of describing a specimen of a free body unusual to this serous cavity has therefore also afforded me the privilege of briefly summarizing the knowledge in the field.

REPORT OF A CASE

A 55 year old white man had on the right side a reducible inguinal hernia of fifteen years' duration. Two days before admission he noticed that the mass could no longer be reduced and experienced pain on pressure over this area. The pain was not relieved by local cold applications or rest in bed.

There was a fluctuant swelling extending from the right anterior superior spine into the scrotum and filling the entire inguinal canal. In the canal was a hard mass, about 2.5 cm. in diameter, and a similar mass could be palpated in the scrotum. Because of the accumulation of fluid in the sac, a definite impulse on coughing could not be felt. The hydrocele of the tunica, extending along the inguinal canal, could not be compressed into the abdomen, suggesting that the internal ring was closed. The preoperative diagnosis was irreducible congenital hernia, undescended testicle and hydrocele of the tunica vaginalis.

At operation a sac 18 cm. long was found containing two segments of omentum, which had undergone torsion. One was deep purplish; the other was hyperemic. The testicle was found at the external ring. The fluid content of the hydrocele was clear and of an amber color. Lying free in the scrotal sac was a regularly oval smooth hard mass.

The postoperative course was uneventful, and the patient left the hospital thirteen days after the operation.

Gross Appearance of Specimen.—The specimen consisted of a hernial sac with a rather small and soft testicle and epididymis, together with a small portion of spermatic cord. There also were some small pieces of omentum showing partial fibrosis. The free body was like hard rubber in consistency, ovoid, measuring 2.8 by 2.6 by 2.2 cm., and of a pale yellowish green, partly light red color. It was hard to cut. In its center a flat ovoid smooth-walled cavity, 1 by 0.5 by 0.5 cm., was situated. This was filled with a light ochre-colored homogeneous and rather soft mass,

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From the Department of Pathology, Beth Israel Hospital.

1. Schmidt, G. B.: München. med. Wchnschr. **80**:410, 1933.

2. Ritter, L.: Deutsche Ztschr. f. Chir. **182**:308, 1923.

Microscopic Appearance.—The free body consisted of a densely packed, almost homogeneous-appearing substance, which at the cut edges separated into parallel layers averaging 4 microns in thickness. The many lancet-shaped clefts which were seen in this substance were obviously artefacts. This material did not take any nuclear stain; it became pale red with eosin and held the fibrin stain rather tenaciously.

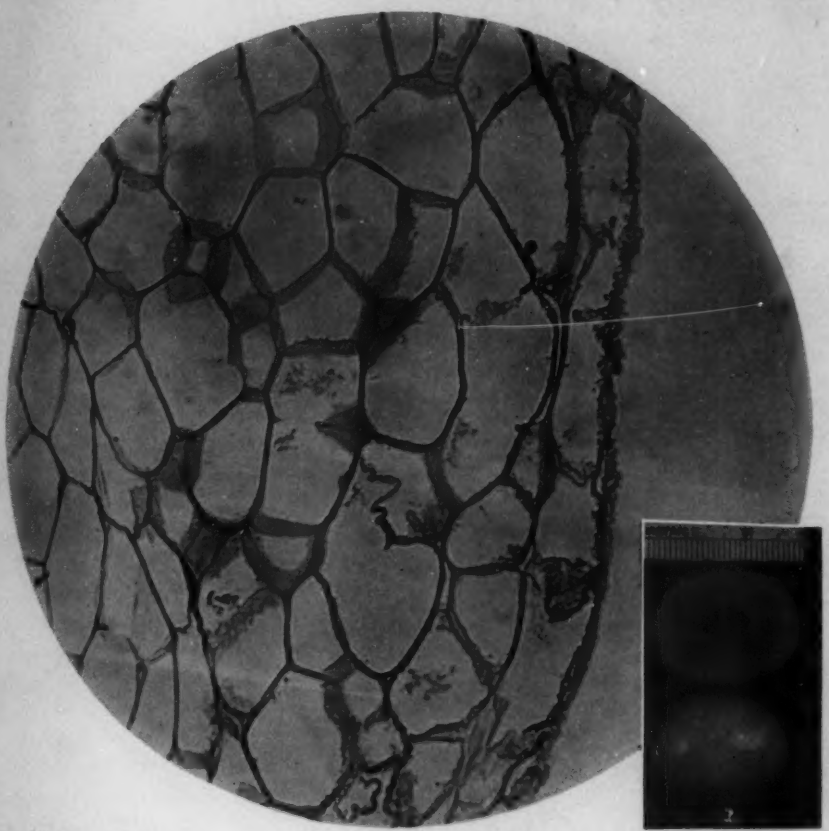

Fig. 1.—The inset shows the free body halved lengthwise. Note the central cavity. The concentric structure is not recognizable. The rest of the figure shows fat tissue from the center of the free body. The structure in general is well preserved.

The brownish soft material from the center was fat tissue, the network of which was well preserved. Occasionally nuclei could be detected, and some erythrocytes were seen in the capillary spaces. The testicle gave the characteristic picture of fibrosis. The epididymis in part showed changes such as are often found in retention of the testicle. There was severe old and recent periorchitis.

[After this paper was written another specimen attracted my attention. In the tunica vaginalis of a 50 year old man who had died of tuberculosis and diabetes was found a dumbbell-shaped concretion, measuring about 3 by 2 by 2 mm. Chemical examination showed calcium phosphate. Histologically, the outer layer presented the same picture as that of the other specimen. In the center, however, partly calcified fat tissue was seen. As figure 2 shows, part of the fat cells had a thin calcific shell or lining, and many of them contained an irregularly round laminated microconcretion.

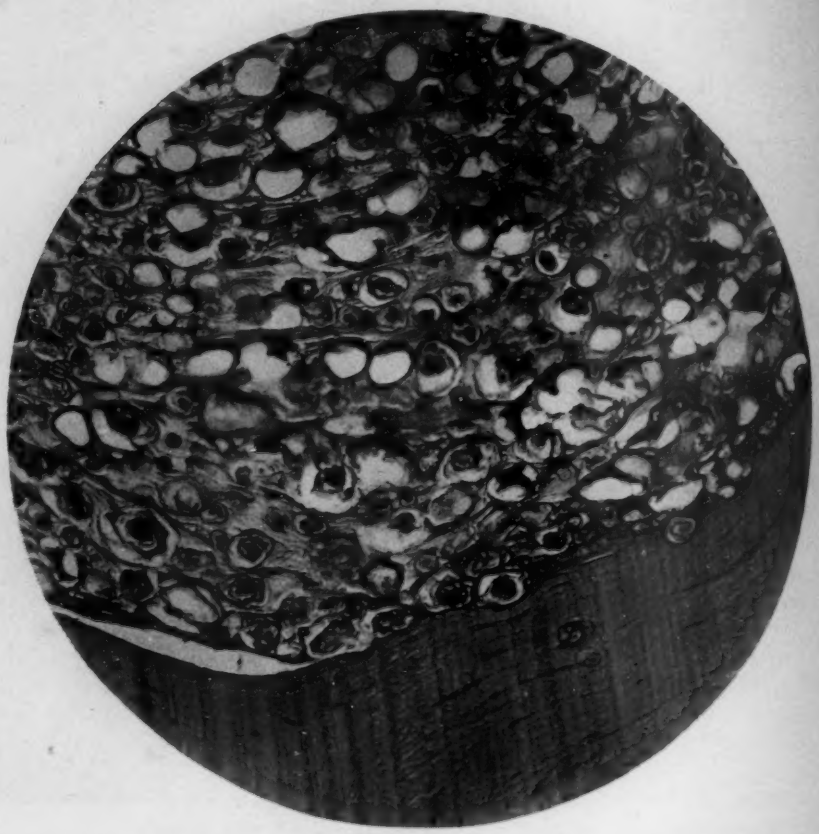

Fig. 2.—Center of a free body, showing fat cells, some of which have thin calcific shells. Many of the fat cells contain each an irregularly round laminated microconcretion.

If continued attention were given to this matter it probably would bring to light a variety of changes in the fat tissue originally forming the nucleus of a free body.]

COMMENT

Most of the free bodies that have been found in the tunica vaginalis have been of small size, ranging from that of a body just macroscopically recognizable to that of a body as large as a pea. Free bodies of

the latter size represent the most common variety, and they may be multiple. Indeed, Sultan [3] found from 1,000 to 1,200 free round bodies, varying in size from that of a buckshot to that of a coffee bean, filling the tunica vaginalis in a case of incarcerated congenital hernia. This is a rare exception, however, from 2 to 5 being the average number of bodies of this size (Ritter).

Free bodies larger than these are much rarer. Oberndorfer [4] stated that they may reach the size of a "plum" and reported one measuring 2 by 2 by 0.5 cm., found in a hydrocele. It was porcelain white and of a cartilaginous consistence, and was made up of concentric layers of anuclear hyaline "connective tissue." A photograph of his specimen shows a central cavity instead of a nucleus, suggesting the possibility that the latter may have been lost in handling. Others have reported such large single free bodies (Chassaignac,[5a] one 2 by 1.2 cm.; Glass,[5b] one 1.4 cm. in diameter; Ritter,[2] one 1.2 by 0.8 cm.; Lavenant,[7] one 1.3 by 0.6 cm.). Apparently, therefore, the specimen I have described is of unusual size, its measurements definitely exceeding those just mentioned.

Various suggestions have been propounded as to the origin of free bodies of the tunica vaginalis. They may have arisen from: (*a*) broken-off bits of tissue in periorchitis proliferans (Virchow) or periorchitis villosa (Klebs); (*b*) detached hydatid bodies of Morgagni (Volkmann; Meyer [8]); (*c*) incrusted epithelial scales (Luschka; Vauthier); (*d*) blood coagulums and fibrin clumps after inflammation (Roux).

Although in most of these theories it has been assumed that a detached piece of tissue serves as the nucleus for the formation of a free body, I have not been able in a reasonably exhaustive review of the literature to find a single report in which the statement is made that a nucleus of histologically recognizable fat or of any other tissue has been found in a free body of the tunica vaginalis. Ritter,[2] Langhans [5a] and Hartmann [5a] each mentioned the finding of fat droplets and cholesterol crystals in the center of the free bodies which they studied, but none reported finding fat tissue. However, identifiable pieces of tissue have been described as composing the nuclei of free bodies in other serous cavities.

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3. Sultan, D.: Virchows Arch. f. path. Anat. **140**:449, 1895.

4. Oberndorfer, S., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 6, pt. 3, p. 736.

5. (*a*) Cited by Ritter [2]; (*b*) cited by Lavenant.[7]

6. Glass, E.: Zentralbl. f. Chir. **47**:266, 1920.

7. Lavenant, M.: Bull. et mém. Soc. de chir. de Paris **20**:819, 1928.

8. Meyer, A. W.: Am. J. Path. **4**:445, 1928.

Owing to the fact that in the case reported here there was an associated congenital hernia, with an internal ring that was estimated by the surgeon as having had a diameter of approximately 2.5 cm., the question of an abdominal free body was thought to deserve consideration. It is theoretically possible that the free body might have originally been formed in the peritoneal cavity and have lodged secondarily in the tunica vaginalis.

Indeed, Hoche [9] in an excellent review of the subject of abdominal free bodies reported the finding of a large one, 5.2 by 4.5 cm., which on section showed a gross and histologic picture almost identical with that described here. In the same necropsy were found three lobules of fat tissue, 1.5 by 1 cm. each, attached to the greater omentum by pedicles and possessing on their outer surfaces lamellae of fibrin showing "fibroid changes." In his discussion of the earlier literature Hoche cited several instances of similar bodies being found in various locations in the peritoneal cavity. Three of these are of interest because of their relation to hernial sacs.

In 1850 Canton [10] described a free body, 5 by 3.7 cm., which showed grossly a fatty nucleus the size of a marble, with an outer shell of concentric thin lamellae about 1 cm. thick. No microscopic examination was reported.

Shaw [10] described a free body which was extracted from a hernial sac "after passage from the peritoneal cavity." It was 3.7 by 3 cm. in size, had a central chalky and fatty nucleus and a shell of "fibrocartilage." Its origin was claimed to be from a detached epiploic appendix. It had no pedicle. No histologic report was mentioned.

Wood [10] reported a free body in a hernial sac, which body in the living patient could be pushed up into the abdominal cavity.

Riedel [11] also presented evidence tending to demonstrate the possible origin of such free bodies from fatty appendages of the large bowel. In this connection it is also interesting to note the experimental production of fat-containing abdominal free bodies by Tomellini,[12] who could cause a free body to develop by tying off an epiploic appendix at its base, if, by means of mild chronic inflammation, he made resorption difficult.

It seems highly plausible, therefore, in the absence of other possible sources for the fat tissue which made up the center of the present specimen, that it may have had its origin in either a detached piece of omental tissue or a broken-off epiploic appendix. The possibility of an intraperitoneal origin must be considered in view of the type of hernia presented and because of the evidence afforded by the literature just cited.

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9. Hoche, L.: Arch. de méd. expér. et d'anat. path. **22**:507, 1910.

10. Cited by Hoche.[9]

11. Riedel: München. med. Wchnschr. **52**:2308, 1905.

12. Tomellini, cited by Morpurgo: Ergebn. d. allg. Path. u. path. Anat. **12**: 252, 1908.

SUMMARY

In the central cavity of an unusually large free body in the tunica vaginalis testis a piece of fat tissue was found. This fat tissue probably came from an epiploic appendix or from a detached piece of omentum.

In most of the instances of free body in the tunica vaginalis the free body has been of small size. Often there have been multiple free bodies. Theories as to their origin usually ascribe their formation to causes within the tunical sac, as there is usually no communication between the sac and the general peritoneal cavity.

General Reviews

ELASTIC TISSUE

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INTRODUCTION

A large part of the human body is composed of tissues which are designed in such a way as to enable them to perform mechanical functions. Some of these tissues may be considered as essentially in an intercellular position. They are usually present in the form of fibrils, membranes or matrices. They are fundamentally organic in composition, although inorganic materials may occur in quantities that are sufficient to obscure the basic structure.

The mechanisms which are operative in the formation, maintenance and disintegration of these tissues are not well understood. The tissues are subject to a wide variety of transformations under the influence of many normal and pathologic stimuli of a local or a general nature. By reason of this behavior they are entitled to an important place in many studies of growth, differentiation, senescence, injury and repair.

The intercellular substance with which this article is concerned is known as elastic tissue. It is selected first for presentation because it has been investigated rather completely from several points of view.

HISTORICAL SUMMARY

The ancients must have been familiar with the elastic qualities of various tissues. Yet, Hippocrates makes no reference to this important property. The extensive, though often erroneous, observations on the pulse and blood vessels made by Galen failed to reveal the importance of the elastic nature of the arterial wall. In the "De pulsibus" he noted degrees of softness or hardness of the vascular walls, which he described as fleshlike or as dry and hard, like leather. Hieronymus Fabricius, one of the greatest of the early anatomists, must not have been aware of the true character of the arteries, or else his pupil, William Harvey,

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failed to listen carefully to his teachings. Harvey came nearest to a conception of the elasticity of blood vessels when he wrote, "Hence, since Nature makes nothing in vain and does the best everywhere, the nearer arteries are to the heart, the more do they differ from veins in structure. Here, they are stronger and more ligamentous."

In the middle of the eighteenth century, Albrecht von Haller, a remarkable scholar and scientist, made the first careful studies and comments on the elastic property of the connective tissues, designated by him as the tela cellulosa. His capacity for perception of basic principles is not more amply demonstrated than in the following excerpts from his dissertation on the tela cellulosa: "The primary simple fibre such as we rather comprehend from reason than sense is composed of earthly particles, adhering longitudinally and connected by intervening and cohesive gluten." Then, after a description of the distribution and nature of the fibers, he returned to a consideration of the interfibrillar substance, gluten: "But here the order of nature seems to be that the fibres above mentioned are all originally formed of this gluten." And in a later paragraph he continued: "It seems then, that an albuminous fluid, with a small portion of earth first concretes into filaments, from some pressure whose causes we now pass over." In regard to the elasticity of the tela cellulosa, he stated: "It possesses a contractile power different from irritability, which though not demonstrable by experiments, disposes the cellular fibre to shorten itself, though for the most part slowly, after having been stretched." This was a close approximation to the truth, but probably the activity of smooth muscle offered a partial explanation for some of the contractile phenomena which he cited.

Although Haller attributed the elasticity of arteries to the circular fibers, Flemyng, his contemporary, more closely approached an accurate understanding of the arterial wall when he wrote: "But when the arteries are fully distended, as the impetus ceases, the distending fluid being no more supplied, they will be left to contract themselves which they do either by the muscular action of the circular fibres or by their natural elasticity or both (but I believe much more by the latter than the former) and so . . ."

The segregation of elastic tissue, collagenous connective tissue and smooth muscle as distinct structural elements awaited the rise of the school of histologists. The founder of histology was Jakob Henle, and to him are attributed the first comprehensive studies on elastic tissue, although Eulenberg and Schwann had made certain minor investigations. Henle (1841) was the first to identify and specify the elastica in the walls of blood vessels. Kölliker (1850; 1861) and Donders were also among the early investigators who sought to throw light on the origin, nature and distribution of elastic tissue. The accumulated observations

as recorded by Kölliker (1850) disclose the eager progress made by the histologists and embryologists. The rising school of pathologists soon began to add contributions dealing with the nature of the elastica in disease. Unna perfected a stain by which the alterations in the character of elastic tissue became more apparent. "Elacin," "elastin," "collacin" and "collastin" became common terms of usage. Strenuous debates arose concerning the origin, deterioration and regeneration of the tissue.

The morphologist, proud of his rapid strides but seeking further aid, introduced the tissue to his colleagues. The physiologist failed to be impressed and never became seriously interested in its functional significance. The chemist added his bit in the form of a few analyses, made principally on the normal bovine ligamentum nuchae. Within recent years there has been greater interest, and as a result the physicist and the chemist have advanced experimental evidence, as well as important theoretic considerations, destined to reveal the molecular structure and arrangement of the elastica. Thus, investigators of this tissue stand, at present, with incompleted tasks in all fields of endeavor, especially in those labors necessary to unify the whole—studies of the function and of the formation and maintenance of the elastica in normal and in pathologic states.

ANATOMIC DISTRIBUTION

Elastic tissue is always in association with collagenous tissue. The proportions of each vary within wide ranges. In general, elastica is less abundant in those places where the connective tissue shows loose texture. Numerous elastic fibrils are found in certain cartilages which are noted for their flexibility and elasticity. Rare fibers may be demonstrated in fibrocartilage. In bone there are a few thick elastic fibers, which usually may be traced into the periosteum. Adipose tissue for the most part has only a scant supply of elastic networks. Above all, the intimate association of elastic tissue with smooth muscle is notable. This is so constant that some authors consider the combination as one tissue—a myoelastic tissue. Here, the anastomosing networks pass between and embrace smooth muscle fibers. In voluntary muscle they form a part of the sheaths and extend between the muscle fibers. Their abundance varies, but they are numerous in the ocular muscles and those muscles which are attached to soft parts, such as those of the tongue and face. Cardiac muscle contains but few fibers.

In addition to the foregoing introduction to the general distribution of elastic tissue, it seems necessary to describe in brief detail the arrangement of elastica in the various bodily systems in order that a more complete understanding of the significance of the tissue in normal and in

pathologic processes may be reached. For much of this description, I am indebted to Maximow and Bloom.

Those systems which are designed chiefly for the mechanical transportation of materials from one part of the body to another are supplied with an abundance of elastic tissue. Among these, the cardiovascular and lymphatic systems are of primary importance. The cardiovascular system may be divided for discussion into the heart, the arteries and the veins, while the lymphatic system may be divided into the spleen, the lymph nodes and the lymphatic channels.

The heart does not have an abundant supply of elastic tissue. The elastica is most prominent in the endocardium, especially in that of the left atrium. From here the elastic networks if traced between the muscular crossbars of the atria are found to be in continuity with the elastica of the epicardium. Although there are fibrils in the endocardium and in the pericardium of the ventricles, they seem to be absent from the myocardium except in the adventitia of large blood vessels. In the annuli fibrosi there are few fibers. The atrioventricular valves contain more elastica in their atrial than in their ventricular halves. The leaflets of the aortic and pulmonary valves have numerous delicate fibrils which extend from the corpora arantii to the annuli, as well as other networks, which are especially numerous beneath the endothelium of the ventricular aspect of each leaflet.

The arteries may be divided into the elastic type, the muscular type, the arterioles and the precapillary arterioles.

The arterioles have a thin internal elastic membrane composed of a network of delicate fibrils. Although there are a few fibers in the adventitia, there is no external elastic membrane.

As the arterioles diminish in caliber to about 62 microns the internal elastic membrane disappears and the adventitia soon loses its elastic network, so that the precapillary arterioles, as a rule, are devoid of elastica.

Arteries of the muscular type have an internal elastic membrane, which in the smaller vessels is an elastic network. In the larger arteries there are subendothelial fibers, and the internal elastic membrane assumes a platelike, fenestrated appearance. This internal elastic membrane may be split into two or three layers, especially where these arteries branch. The increase in the size of these vessels is accompanied by an increase in the number of elastic fibers, which embrace the smooth muscle cells of the media. In the intermediate muscle layers, the elastica may form fenestrated concentric bands, which alternate with bands of muscle fibers. This medial network, which is continuous with that of the intima, fuses externally with the elastica externa. From this condensed layer, fibrils pass outward to enmesh the tissue elements of the adventitia.

Large arteries of the transitional musculoelastic or elastic type have a variable distribution of elastica. As a rule, there are subendothelial fibers which unite with a thick fenestrated internal elastic membrane that in adult life may be composed of several lamellae. The media of these arteries is composed largely of elastic tissue. In the aorta this consists of from fifty to sixty-five concentrically laminated thick elastic membranes. These are joined by delicate elastic fibers and are separated for the most part by collagenous connective tissue and smooth muscle fibers. There is no elastica externa, but in the outer layer of the media are found abundant networks, which anastomose with the rich supply of elastic tissue in the adventitia.

Many variations from the model arteries have been described. The media of the visceral arteries may be composed of an internal muscular coat and an external elastic layer. The cerebral arteries have a well developed elastica interna and very little elastica in the media and adventitia. In the renal arteries the networks are unusually prominent. The coronary arteries have internal and external elastic membranes. Although the inner layers of the media of the coronary arteries are rich in elastica, the outer portions are composed principally of smooth muscle. Other variations in arteries, which accompany certain physiologic and pathologic changes, will be discussed elsewhere.

Veins.—Veins may be divided into those of small, medium and large caliber. There are great variations in the structure of veins and in their corresponding content of elastic tissue. They are much less elastic than arteries, and their elastica is less well developed. In veins larger than 200 microns in caliber, elastica may be found. This is composed of delicate networks and is found in the media and adventitia. In the veins of medium caliber (from 2 to 9 mm.) a few inconspicuous elastic fibers may be distinguished in the intima, and a network of thick longitudinal fibers occasionally is found between the intima and the media. This network never assumes the form of a fenestrated membrane. In certain vessels, such as the saphenous vein, the innermost layer of the media is relatively rich in elastica. In larger veins the external layer of the media has flat networks of longitudinal elastic fibers which often separate the circular muscles into layers. These networks are connected with the fairly prominent meshes of adventitial elastica.

The veins of large caliber usually have a few fibrils in the intima and media, while thick prominent fibers are numerous in the connective tissue of the broad adventitia. Smaller fibers are found in the inner muscular layer which lies adjacent to the media or sometimes next to the intima, if the media is absent.

Each valve of a vein contains a rather thick network of fibers on the side which is directed toward the lumen. These fibers are continuous with the intimal elastica.

Lymphatics.—Elastic fibers occur in the walls of all lymphatics which are of sufficient size to have valves and smooth muscle in their walls. In lymphatics with a diameter greater than 2 mm. there are longitudinal interlacing fibers in the intima, delicate intermuscular fibrils in the media and in the adventitia a moderate amount of elastica, which for the most part is disposed tangentially. The thoracic duct has several intimal layers of delicate fibrils which are directed longitudinally. Near the junction of the intima and media an internal elastic membrane is formed by condensation of the fibrillar network. From this structure anastomosing fibers penetrate the media and join with the thick longitudinal fibers of the adventitia.

The lymph nodes are poorly supplied with elastic tissue, but in the capsules and occasionally in the trabeculae delicate fibrils may be found.

The spleen contains an abundance of elastica. It is especially plentiful in the capsule, where many fibrils, as well as elastic membranes, may be found. These are usually most numerous in the inner layers of the capsule, whence they are continued into the trabeculae, where elastica often exceeds collagenous connective tissue in amount. There are rare fibers in the reticulum of the white pulp. These are most numerous around the central arteries and at the periphery of the malpighian bodies. There is no elastica in the red pulp. As soon as arteries pass into the red pulp, they lose their complement of elastic tissue.

Skin.—The elastic tissue of the integument has been studied thoroughly. The hypodermis has but few fibers. In the derma thick networks ramify independently of spaces between the bundles of collagen. They tend to condense around the adnexa. An interesting arrangement is the attachment of arrectores pilorum muscles to networks of elastica. Beneath the epithelium and in the papillae, numerous delicate fibers form a continuous network. The elastica of the cheek differs from that which occurs elsewhere. It is composed of a dense feltwork of closely arranged twisted fibers, which lies just beneath the epithelium. In general, where the skin folds easily, the elastica is scanty. Where the skin is bound closely to the underlying structures, the fibers usually are more thick and numerous.

Respiratory Tract.—Elastic tissue is found throughout the extent of the respiratory tract. Sparsely distributed delicate fibrils are found in the nasal mucosa. In the larynx, the arytenoid cartilages, although hyaline at their bases, have numerous elastic fibers in the matrix of the uppermost portions. The true vocal cords are composed largely of bands

of elastic fibers. In the lamina propria of the trachea many delicate networks are found. The elastic fibers are so closely arranged around the tracheal cartilages that a compact membrane is formed. The smooth muscle fibers which pass between the free ends of the incomplete cartilaginous rings are inserted principally into dense bundles of elastica which surround the trachea and its cartilages. The lamina propria of the bronchi is richly supplied with elastic fibers that are continuous with the abundant fibrillar network which enmeshes the smooth muscle cells of the bronchial walls and extends throughout the enveloping connective tissue, tending to condense around bronchial cartilages. In the respiratory bronchioles (less than 5 mm. in caliber) the myoelastic membrane is very prominent. Many closely arranged straight thin fibrils are found in the walls of the alveolar ducts and around the openings into the alveolar sacs. The interalveolar septums have a compact meshwork of reticular fibers, but the elastic fibers are few. The visceral pleura contains several prominent layers of elastica. These course at various angles to the plane of the surface.

Alimentary Tract.—The alimentary tract, although well supplied with smooth muscle, has much less elastic tissue than the respiratory tract. The mucous membrane of the mouth contains elastic networks which, except for a larger number of delicate fibers, are similar to those in the skin. In the mucous membrane of the cheek, as well as in the derma, there is an unusually abundant supply of elastica. The soft palate has dense networks, which lie between the lamina propria and the mucous glands of the submucosa. However, on the nasal side of the soft palate similar arrangements of elastica separate the mixed glands of the lamina propria from the muscle. No elastic fibers are found in the periodontal membrane. Beneath the epithelium of the tonsils there is a moderate amount of elastica, which is continued into the cores of the tonsillar folds. In place of a muscularis mucosae the pharynx has a thick layer of fibers, which principally are disposed in a longitudinal direction. Where the pharynx merges with the esophagus, the elastica becomes thinner as the networks are succeeded by the muscularis mucosae, which retains a few delicate fibers. In the fornix the layer of elastica blends into the periosteum of the skull. Throughout the lamina propria delicate fibrils are found both in the pharynx and in the esophagus. The latter has, in addition, coarse networks which ramify throughout the submucosa. The stomach has very little elastica in its wall, especially in the lamina propria. The intestine has a few delicate networks which surround the vessels in the lamina propria and accompany the smooth muscle of the muscularis mucosa. Although the networks increase in prominence in the submucosa, the muscular layers contain only a few fibrils.

Urogenital System.—The kidney has no elastica except that which is associated with vessels. It may be noted that the afferent arterioles have a delicate elastica interna, which disappears with the branching of the vessels to form glomerular arterioles. The efferent arterioles have no elastic membrane. The lamina propria of the renal pelvis, ureter and bladder has a few delicate networks, which are continuous with the fairly abundant elastica of the tunica muscularis. The lamina propria of the urethra is rich in elastic networks.

The genital tract of the male contains much elastic tissue, while that of the female is less well supplied. Elastic nets are present in the tunica albuginea, mediastinum and septula of the testis. The singular basement membrane of the seminiferous tubule contains many very delicate fibrils. Elastica is prominent in the corpora cavernosa, not only between the cavernous blood channels but also in the tunica albuginea. The ovary has no elastica in the cortex, but there are numerous networks in the medulla. The fallopian tube has a few fibers in the muscularis. In the uterus there is no elastica in the endometrium or in the subjacent muscle. Fibers are present in large numbers in the external portions of the uterine musculature, in the cervix and in the vaginal wall. The lobule of the breast normally has no elastica. In the lactating gland elastic fibrils may be found, especially around the excretory ducts.

Biliary and Pancreatic Ducts.—The biliary system has a small amount of elastica around the intrahepatic ducts. The tissue increases in rough proportion to increase in caliber of the ducts. The largest number of fibers are found in the walls of the extrahepatic ducts and in those of the gallbladder. In the latter they tend to accompany the smooth muscle bundles, only a few fibrils being found elsewhere.

The pancreas has only a few delicate networks. These are located around ducts.

Osseous System.—The periosteum contains a few networks, which are more prominent in the external layer. From this layer thick fibers occasionally may be traced into the cortical bone. A few fibers are present in the intervertebral disks and in the capsules of joints.

Central Nervous System.—Except that present in blood vessels, the only elastica in the central nervous system is confined principally to a few networks in the dura and leptomeninges.

Eye.—The eye has a plentiful supply of elastica. The delicate networks of the sclera and those of the choroid join to form the rich meshwork of the lamina suprachoroidea and lamina cribrosa. In the substantia propria of the cornea a series of very delicate networks are found. These are more easily found anterior to Descemet's membrane. Although this membrane resembles elastica in staining qualities, the reactions are not typical. Beneath the pigment epithelium there is a narrow zone of

delicate fibrils. There are numerous fibrils in the ciliary body, but none is found in the uvea. The eyelids have abundant networks.

Ear.—The auricle of the ear has an irregular plate of elastic cartilage, about which there are dense elastic networks. Although the tympanum is composed principally of collagen, delicate elastic fibrils are present, most prominent in the central zone. The eustachian tube is enclosed partially by elastic cartilage.

EMBRYOLOGY

It would be impossible to discuss the complete embryologic evolution and development of the elastica in this limited space. Therefore, it seems appropriate to dwell briefly on the tinctorial qualities of the elastica as it matures, the time and site of its first appearance in certain vertebrates and its distribution in developing chick and human embryos.

The vascular system is the first bodily part to be supplied with elastic tissue. The larvae of certain Amphibia, namely, the salamander (Flemming) and the axolotl (Spalteholz), have been studied. In the former, when it is 3 to 4 cm. in length, elastic fibers appear in the *Mesenterialwurzel.* In the latter, when it is 9 to 10 mm. in length, they may be found in the truncus arteriosus. Among the Aves, Spalteholz demonstrated elastica in the three day chick embryo and the four day duck embryo. In the human embryo elastica first makes its appearance during the third or fourth week, at which time it is found in the aorta (Röthig). The development of the elastica in the chick embryo was studied by Nakai. During the fifth day, two days after the heart begins to beat, fibrils may be found at the base of the aorta and the pulmonary artery, in the peripheral layer of each vessel. By the ninth day, elastica is demonstrable in the epicardium, and about the tenth day it appears in the tissues around joints and insertions of the extremities. By the fourteenth day it is distributed throughout the interstitial tissues but does not appear in the organs until later.

The study of human embryos has been concerned chiefly with the vascular system, lungs and skin. Röthig demonstrated elastica in the aorta during the third or fourth week. Hewer found that fibrils in the cerebral and coronary arteries were well developed as early as the sixteenth week. However, even at the time of gestation certain vessels, notably those of the adrenal, pituitary and thymus, and veins of the renal cortex were supplied with very little elastica. Linser found that the networks in the pulmonary vessels were demonstrable as early as the tenth week but that they did not gain their full fetal complement until the middle of the fifth month. This distribution is changed after birth. Within a period of two or three months of extrauterine life the veins show a relative increase and the arteries a relative decrease in the amount of elastic tissue—thus reaching a stage comparable to that found

in the adult lung. In the course of the third month of embryonic life a thin refractile membrane appears beneath the endothelium of the brachial artery. This does not stain electively until the fourth month; nevertheless it is the anlage of the mature elastica interna (Maximow). During the fourth month the elastica externa makes its appearance. Coincident with the increase in the number of smooth muscle cells in the media, an increasing number of elastic fibrils develop. Thus, the medium-sized arteries, of which the brachial is an example, attain their complement of networks.

Linser investigated the development of elastica in the lungs of human embryos. Early in the fourth month fibrils appear along the larger bronchi. These increase in number and assume a purposeful laminate arrangement, so that at the beginning of the fifth month there are six to eight delicate bands beneath the epithelium. By this time elastic networks are forming among the smooth muscle bundles as well as in the peribronchial tissues. In the latter situation they tend to condense around the cartilages, while only rare fibrils are found in the cartilages. During the fifth month a few fibrils appear in the lung parenchyma, especially along small vessels and adjacent to epithelial-lined structures. It is not until the seventh month that fibrils are distinguishable in the stroma of the parenchyma. By the end of the seventh month the elastica reaches the full state of antenatal development. During the first month of extrauterine life the elastica becomes more abundant and reaches the state of differentiation which is found in the adult lung.

The progressive stages of differentiation of the pulmonary elastic tissue are analogous to those which were described by Maximow as occurring in the internal elastic membrane of the brachial artery. The elastic fibrils are at first only weakly stainable by the specific stains. The intensity of the staining reaction increases gradually, but with unusual rapidity during the sixth month. But, surprising though it may seem, the full depth of staining, which corresponds to that of the vascular elastica, is not attained until after several days of extrauterine life. The observations of Sudsuki and Röthig in general were in agreement with those which have been described.

Linser found that the development of the elastica in the lungs of a number of mammals was similar to that which occurred in man. The chief difference was in the rapidity of formation. As a general rule, he found that the more active the animal the more rapid were the formation and maturation of elastic tissue.

The development of elastic tissue in the skin has been studied in detail by Lynch. It appears in the blood vessels at the fifth month and in the corium during the sixth month. According to White, there is a great increase in the amount during the eighth month. Hewer found an abundance of elastica in the skin at the fourth month. It may be

that certain discrepancies are explicable on the basis that there is a great variation in the amount of elastica from place to place.

Hewer attempted to correlate the time of appearance of elastica in the human embryo with the beginning of functional activity of tissues and organs. In the study the basement membranes apparently were considered as a part of the elastic tissue system. The first appearance of elastica in the kidneys was in the interstitial tissue at eight weeks. At sixteen weeks there was a definite basement membrane to Bowman's capsule, the convoluted tubules and Henle's loop. Previously, during the sixth to the eighth week, the ureter had gained a basement membrane. The author assumed that the kidney began to function at about the twelfth week. Elastica was found in the spleen as early as the twelfth week and was present in abundance in the skin by the sixteenth week. In contrast to the vascular system, lungs and skin, the alimentary tract showed a scanty and much retarded development of elastica. A basement membrane appeared beneath the epithelium of the esophagus during the twelfth week. There was no elastica in the small intestine. A few fibrils were found in the muscularis mucosae of the stomach, cecum and appendix after the sixteenth week. The parathyroid contained no fibrils. By the sixteenth week the thyroid follicles had been supplied with a basement membrane and the glandular stroma showed numerous fibrils. The elastica began to develop in the stroma of the adrenals at eight weeks and in the pituitary at thirty-two weeks. The tunica albuginea, trabeculae and basement membranes of the testis showed some elastica at eighteen weeks. As early as the sixth week fibrils began to appear beneath the germinal epithelium of the ovary, and a few fibers penetrated the stroma. No elastica was found in the thymus. By the sixteenth week there was an "elastic" basement membrane of the choroid plexus. At this time there were a few delicate fibrils in the pia-arachnoid. After this thorough study, Hewer was unable to arrive at definite conclusions concerning the influence of function on the origin and maturation of the elastica. The chief difficulty was the lack of knowledge concerning the time of onset of function in most of the organs and tissues.

PHYLOGENY

Exact information concerning the occurrence and distribution of elastic tissue in the invertebrates and lower vertebrates is meager. From the accumulated data, one might believe that what occurred phylogenetically in the animal series is analogous to that which occurs ontogenetically in man.

In the invertebrate series, studies have been made on certain Mollusca and Chordata. Argaud (1909) stated that there were no true elastic fibers in Mollusca, but there were fibrils which had the physical character-

istics without the staining qualities of elastica. In Arion, Argaud (1909) found no fibrils of this nature. In Eledone moschata, beneath vascular endothelium there was a hyaline limiting membrane which had the optical but not the staining reactions of elastic tissue. Inasmuch as A. Aschoff (1893) stated that the first anlage of the elastica interna in the human embryo appeared as a hyaline membrane, Argaud raised the question as to whether this hyaline membrane could be connected phylogenetically with the hyaline membrane of Mollusca. Schiefferdecker studied Sepia officinalis but could find no elastic fibers stainable as such. Wetekamp found in the typhlosole and in the stomach wall of Anodonta cellensis not only connective tissue but also elastic fibrils. Indeed, the whole intestine seemed to be enclosed in an elastic net. Schiefferdecker was unable to demonstrate elastic fibrils in the typhlosole of Anodonta and Unio. Among Chordata, Bütschli studied the amphioxus and found elastic fibrils in the perichordal tissues. Schiefferdecker could not confirm this.

Observations relative to the vertebrate series were less contradictory. Two Cyclostomata, Petromyzon marinus and Petromyzon fluviatilis, were studied. Argaud (1908) found no elastica in the former. In the latter, Schiefferdecker noted around the chorda a membrane which resembled elastic tissue. In two Ganoidei, Acipenser sturio and Acipenser ruthenus, there were well formed typical elastic fibers, especially in the walls of blood vessels, around the chorda and along the vertebral column (Schiefferdecker). In two Selachii, Scyllium stellare and Torpedo ocellata, Schiefferdecker found an abundance of elastica through the body. The same has been found to be true for all higher vertebrates. The amount and distribution of elastica differ widely, however, as may be inferred from the careful descriptions of the pulmonary elastica of various Amphibia, Reptilia, Aves and Mammalia (Ogawa).

From the accumulated observations it seems that in the lower animals there is a tissue which has the physical properties but not the staining reactions of elastica. Higher in the animal scale, in Petromyzon, elastica tissue which can be differentiated by staining reactions first appears. Even in this instance, the staining qualities are not quite typical. In Ganoidei characteristic elastica makes its appearance. Phylogenetically, the early appearance of elastica and the assumption of the differential staining reaction are comparable to those developmental stages of the elastica in man. In the latter instance, first the fiber is refractory to the stain, secondly the fiber is impregnated weakly, and finally the fiber assumes full differential staining qualities. In this regard it is of interest that a similar development of morphologic and tinctorial properties was described by Bloom, who studied the formation of elastic fibers in tissue culture.

Finally, the reversal of the developmental sequence occurs in certain regressive changes which will be considered in the discussion of the pathology of the elastica.

HISTOGENESIS

Controversial observations and spirited polemics have marked the progress of knowledge of the mode and site of origin of the elastic elements of the connective tissues and cartilage.

Before the numerous theories are discussed certain fundamental questions which bear on all hypotheses must be considered. First, does the elastic fibril originate by coalescence of granules or is it developed as a continuous delicate fibril? Second, if it develops as a fiber, is it from the beginning a fully differentiated fiber, or is it formed, by means which for the present one may disregard, from a preelastic fibril, from an undifferentiated fiber or from a collagen fiber?

The consensus is that the elastic fiber, whether it is in cartilage or elsewhere, first appears as a continuous fibril. Evidence to the contrary has been advanced by Ranvier, Gerlach, Deutschmann, Loisel, Gardner and de Kervily. Ranvier held that the fibers were formed by a fusion of granules. Gerlach and Deutschmann demonstrated granules, which were bound alongside cells. They contended that these were the precursors of the elastic fibril. Loisel believed that the elastica arose as a fiber derived from the cell protoplasm, but in the intercellular matrix he noted elastic granules the purpose of which seemed to be to augment the size of the formed fiber. Gardner found in the protoplasm of cells tiny granules, which became alined so as to form intracytoplasmic fibrils by coalescence. De Kervily showed by silver stains that a large number of fibrils, especially in the cartilages and perichondrium of the respiratory tract, appeared to be composed of rows of small granules.

Other authors were content to consider the original structure as a fibril, but they failed to agree as to the exact nature of the fiber. Loisel, Linser and von Korff contended that the product of the fibroblast was an undifferentiated fiber which could be transformed into either collagenous or elastic elements. Virchow (1851), Schiffmann, Gugot, Fuss and Krösing (see Röthig) believed that elastic fibers arose by alteration of the character of the collagenous fibril. Other authors expressed the opinion that the elastic elements were formed independently and from the beginning were destined to be elastic tissue.

Further questions which faced the investigators may be outlined as follows: Does the elastic fiber originate from cell protoplasm or from the intercellular matrix? In either instance, is the formation dependent on specific cellular activity? If cellular activity is of fundamental importance, is there a specific cell whose sole function is the elaboration of the elastic fibril? What is the mechanism by which the fiber is controlled

after it has gained an intercellular position? What part do mechanical or other forces play in this mechanism?

For purposes of correlation one may take the liberty of classifying the results of many studies into three groups. There were those authors who believed in the cellular origin of the elastica. Their contemporaries advanced a contradictory hypothesis which concerned itself with the formation of fibrils in the intercellular substance without specific participation of cells. The third group of observers combined the aforesaid cellular and intercellular theories.

The cellular theory of origin includes all points of view which are concerned with fibril formation by virtue of cellular activity from nuclear material, intracytoplasmic granules, cytoplasm, ectoplasm or secreted substances. Advocates of this theory were Schwann, Henle (1841), Boll, Hertwig, Gerlach, Fol, Taddei, Nakai, Spalteholz, Jores (1907), Ladwig, de Kervily, Orsos (1926) and Krompecher (1928).

The second, or intercellular, theory was supported by Kölliker (1850), Henle (1852), Schwalbe, Weismann, Rabl-Rückhard, Kollmann and Fuss.

The third theory was developed on the basis of a new conception of the fundamental nature of the intercellular ground substance. Hansen, Spüler, Loisel, Mall, Flemming, Geipel, Meves, Hueck and Ladwig supported this hypothesis.

Even within the separate groups there were variations of opinion. For this reason it seemed worth while to present briefly the ideas of certain authors.

Henle in his earlier writings regarded elastic fibers as originating from the nuclei of connective tissue cells, although subsequently he was inclined toward the point of view that they arose in the intercellular substance.

Hertwig found that the fibers first appeared along the margins of cells. He believed that they were constructed from the protoplasm by specific activity of the cells from which they arose.

Gerlach contended that the fibers, at first very delicate and difficult to distinguish, were formed at the cell surfaces and later were separated from the cell.

Fol proposed the theory that elastic elements in cartilages were never a differentiation product of the cytoplasm but assumed fibrillar form in the coagulum of the cell secretion.

Taddei agreed with the conception that young elastic fibers were differentiated in the protoplasm of connective tissue cells and eventually were cast off into the intercellular spaces.

Nakai was able to follow the stages by which the processes of mesenchymal cells were converted into elastic fibrils.

Jores (1907) supported the contention that elastic fibers developed from cell protoplasm without passing through an intermediate connective tissue fiber stage. He arrived at these conclusions after a series of careful studies.

Ladwig agreed with those who believed that elastic fibers were differentiated along the periphery of fibroblasts.

Spalteholz believed that his studies favored the intracellular theory of origin.

De Kervily demonstrated in certain cartilages of human embryos fusiform cells which contained protoplasmic granules that had an affinity for silver. He believed that these granules coalesced and in combination with the cell protoplasm gave rise to the elastic fibril. Also, he demonstrated intracellular granules which stained electively with elastic tissue stains. He assumed that these were the basic substances of the elastic fibril.

Orsos (1926) found in the same cell two types of fibrils, having different tinctorial and optical properties. The staining reactions indicated that one type was composed of an albuminoid substance and the other of globulin. The former gave rise to collagen fibrils, and the latter became differentiated into the "elastica."

Loisel and Krompecher were not content to accept the majority opinion that the fibroblast or undifferentiated connective tissue cell elaborated the elastic fibril. They presented evidence in support of a theory that the fibril formation was restricted to a specific type of mesenchymal cell, which had no power to form other types of fibers. Loisel designated this cell as an "elastoblast." Both observers believed that this cell could be recognized by its distinctive histologic structure. Krompecher believed that in pathologic as well as in physiologic states the elastic elements were formed along the borders of the elastoblasts and that as soon as the elastic elements were differentiated the specific cells tended to disappear and no new fibers developed unless there was a regrowth of elastoblasts.

The few authors who believed in the origin of the elastic fibril solely from the intercellular matrix did not undertake the complete studies that were made by those who advocated participation of the cell in the formation.

Kölliker (1850) and Schwalbe agreed that the elastica was formed by a peculiar transformation of the intercellular substance of the connective tissue anlage. They believed that all large fibers developed by an increase in size of the small delicate fibrils.

Rabl-Rückhard contended that the elastic fibrils in the cartilage of the ear were formed not from the cells but by differentiation of a part of the hyaline ground substance.

Weismann and Kollmann believed that formative powers were present in the intercellular substance, by which the fibrils were developed.

Matsukoa attributed the new formation of elastic tissue in regenerating cartilage to a morphologic and chemical change of the intercellular substance.

Fuss stated that the cells had no direct part in the formation of fibrils but that these arose from fibers which were chemically identical with collagenous fibers.

The blending of divergent opinions found expression in the conclusions of several authors who gave due consideration to the part played not only by the cell but also by the intercellular materials.

Spüler expressed the opinion that elastic elements were formed by the cells. He believed, however, that the formation was not necessarily dependent on the cell body but that the development of the fibril could occur at a distance from the cell in the ground substance. In 1897 he stated that the outer zones of the cell were a part of the ground substance. These zones seemed to have an inherent formative ability and could readily be split off from the cell.

Loisel believed that certain specific cells, the elastoblasts, formed fibrils at the expense of their processes and their protoplasmic periphery. These fibrils became isolated in the form of a protoplasmic spindle enclosed by a fibrillar mantle. After separation from the cell most of the fibers assumed the character of elastic fibers, and the remainder became a part of the connective tissue. Furthermore, in the isolated protoplasmic part, elastic granules appeared. Their purpose seemed to be for the augmentation of the elastic fibril. Later, the growth of the fiber seemed to occur through a transformation of connective tissue substance into elastic substance.

Hansen agreed with the belief that there was a transformation of protoplasm and protoplasmic processes into elastic fibrils, but he concluded that in cartilage the fibrils were developed from the intercellular matrix.

Mall divided the protoplasm of the mesenchymal syncytium into endoplasm and ectoplasm. He believed that elastic and collagenous fibers arose in the ectoplasm and that one cell was concerned in the formation of both tissues. Flemming also believed that the fibrils took form in the ectoplasmic mantle of cells. He contended that this mode of origin would explain the apparent development of fibers in the intercellular matrix of cartilage.

Geipel concluded that the elastica was formed in the cell sheath or mantle and that it was a matter of individual measurement whether one might consider the fibers as a constituent part of the cell or as a part of the earliest deposit of the intercellular substance.

Meves maintained that the intercellular substance had an inherent ability to produce new fibrils.

Hueck supported a belief which is concerned with a ground substance formed by a thickening of the protoplasmic margins and a continuous recurring separation of these parts from the protoplasm so as to form an undifferentiated intercellular substance from which elastic and collagenous fibrils may arise.

Ladwig on the basis of heterotransplantation experiments concluded that elastic fibrils differentiated along the margins of fibroblasts.

Bloom found that the elastic fibers which developed in tissue cultures of guinea pig heart muscle were always extracellular in position.

It seems logical at this point to consider briefly a few factors which may exert an influence on the origin and augmentation of the elastic fibril. These may be evolutionary, physical, chemical, hereditary or hormonal. The hereditary, the hormonal and to a certain extent the chemical factors will be discussed in the part of this review which deals with the pathology of the elastica. The phylogenesis of elastic tissue and its recapitulation in the embryologic development of the human embryo have been discussed under the appropriate headings.

Physical factors of stress and strain were believed by His to be of great importance in the development of connective tissue structures. Roux, a leader of the "mechanistic school," contended at one time that function and action were the sole influences which initiated and guided the development of the connective tissue structures. Later, he admitted that this idea failed to explain the intricate anatomic character of certain connective tissue elements. He classified these structures as nonfunctional and hereditary. Despite the difficulties of actual proof, he contended that there were two great stages of development. The first period included the embryonic state, during which time the constituent parts appeared, differentiated and grew by virture of inherent qualities. The second period was characterized by a more complete development of the various parts under the guiding influence of stimuli.

Melnikow-Raswedenkow favored the theory that elastic tissue normally made its appearance where there was a mechanical necessity. Jores (1902) gave certain good reasons for his opposition to this point of view. Scagliosi in instances of phlebectasia described new formation of elastic tissue in those sites where the wall of the blood vessel had been weakened by medial degeneration. Maximow and Bloom concluded that in general the amount and degree of development of the elastica in the wall of the blood vessel were more or less proportional to the pressure of blood within the vessel. Bloom found that elastic fibers were formed in tissue cultures of embryonic guinea pig heart muscle. He stated that although contractile pulsation of the

muscle explant was not essential for their formation, they attained their greatest development under its influence. Thus, what investigators have learned of the phylogenesis, embryonic development, normal anatomic distribution and in vitro cultivation of elastic tissue tends to support the hypothesis that mechanical forces may exert an influence on the genesis and development of the elastica, especially if those forces are rhythmic and fluctuating.

In the preceding accounts, a discussion of the participation of a theoretical chemical substance, elastin, in the formation and maturation of elastica has been omitted. Ranke (see Hueck) and Hueck were two of the several authors who advocated the hypothesis that the fibers which are formed may be impregnated with either collagen or elastin and that this impregnation will determine the ultimate nature of the fiber.

Bierich believed that certain alterations in the physicochemical condition of the collagen fibers fitted them for impregnation with elastin. The more or less variable and interchangeable staining reactions of collagen and elastin in various pathologic states have induced many authors to accept the stated hypothesis. Unna (1928) has discussed the possible chemical nature of these substances.

Further indication that elastin is a specific chemical substance with which fibers may become impregnated has been advanced by tissue culture studies. Erdmann found that elastic fibrils, as judged by staining reactions, were formed in tissue cultures only in those instances in which elastin was present in the tissue prior to explantation. Bloom found that in cultures of embryonic aorta the elastic fibrils which developed took form near the ends of the explant and seemed to be continuous with the preformed elastica in the media. Odiette agreed with the observations of Erdmann. He was able to influence the in vitro development and degeneration of elastic fibers by using various combinations of amino acids in the culture medium. Therefore it seems, from the accumulated facts, that future investigations will be concerned with a substance or substances which are elaborated from relatively simple chemical compounds and deposited in the elective sites. It may be predicted that such substances will be of such a labile nature as to be resorbed, redeposited and augmented in response to certain physiologic stimuli and as a result of certain pathologic processes.

From the foregoing considerations it may be concluded that:

1. Maturation of the elastic tissue morphologically and tinctorially is a relatively slow process.

2. Ontogenetic development of the individual fibril in man simulates the development which occurs phylogenetically.

3. Fibril formation, if influenced by stimuli in the same way as the collagenous tissue, does not respond in a like manner either as to time, place or degree.

4. Embryonic sequences indicate that although the structure and distribution of elastic tissue may in part be predetermined, functional demands, beginning in early embryonic life, also play an important role.

5. The sequences in the development of elastic fiber networks, if one adheres to a cellular theory of origin, remain obscure even though one accepts the postulate that there is a continuous change of endoplasm into ectoplasm.

PROPERTIES

The physical and chemical properties of elastic tissue differ from those of collagenous connective tissue. In treating of this subject the terms "elastin" and "collagen" will be used frequently. These terms are merely names given to the material which composes each type of tissue.

The exact role of each tissue in response to the application of force is unknown. Certain inferences have been made and handed on from one generation to another without proper justification. Within certain limits, after deformation these tissues promptly resume their original form. Inasmuch as one type of tissue is usually accompanied by the other in varied proportion, arrangement and compactness, the part played by each tissue or other elements with which they commonly are associated in the resumption of form has not been studied adequately. Be that as it may, certain observations have led most authors to believe that the elastic tissue has, in the common understanding of the term, great elasticity, while collagen has little inherent power of resumption of form after great deformation. Although knowledge is meager, by an analysis of the physical and chemical nature of elastic tissue certain pertinent questions may be approached. First, how is the tissue designed? In answering this question, the anatomic distribution throughout the body, the microscopic structure of the constituent parts and the more minute physical structure deserve consideration. Secondly, is this design of such nature that it fulfils the requirements by which materials become possessed of great elasticity? In this regard certain laws of elasticity, both physical as applied to rods and wires and physicochemical as applied to elastic colloids, will be considered.

Physical Properties.—The elastica may occur in the form of circular fibrils, as flat bands or as membranes which often are fenestrated. The tissue in yellow. As a rule, it is composed of fibrils which form continuous networks. Normally, no free ends can be found. The unit fibers, band or membranes vary in diameter from almost ultramicro-

scopic dimensions to between 10 and 12 microns. When they are under normal tension, they tend to be straight. If this tension is reduced, they assume an undulate, spiral or angulate form. If a greater than normal force is applied, they exhibit extraordinary extensibility, and as soon as the deforming force is removed, they have an inherent capacity for returning quickly to almost their original shape. At the limit of extensibility they usually rupture in a transverse direction, without splitting, and the broken ends tend to curl in a spiral manner. The extensibility is greater in the long axis of the fiber than in a direction perpendicular to the axis (Wohlisch). In their natural state they are highly refractile and almost isotropic. With drying or stretching they become doubly refractile. While they are drying there is a great reduction in thickness but very little proportional diminution in length (Wohlisch). The fibrils are paramagnetic and exhibit electric and magnetic anisotropy. If one assumes that basophilic matter is on the positive pole and acidophilic matter on the negative pole, elastic tissue is positive to collagenous tissue, and a difference in electrical potential must exist between the two.

There is little similarity between the thermoelastic characteristics of this tissue and those of rubber, with which it has been compared so frequently (Wohlisch). As the temperature is increased from 0 to 60 C. the fibrils soften, gradually diminish in length and increase in thickness. Up to 60 C. these changes are reversible. Above 60 C. the fibers become hard, and there is a decrease in volume, which is evidenced by a diminution in length and breadth.

Theoretic conceptions of the fundamental physical structure of the elastic fiber have been advanced on the basis of data obtained by roentgen ray spectrographic analysis by Herzog and Gonell. These observers concluded that collagenous and elastic fibrils were composed of regularly oriented microcrystals. From their observations, if fiber diagrams of roentgen ray diffraction patterns may be used as a source of authority, it may be contended that the main valence chains of the elastic fiber must extend in the long axis of the fibril, while weak or side valences are assumed vertically. The main valence chains would be grouped to form micellae, which may be considered as the primary units of the fiber. The data which are presented by them are not complete or entirely convincing. Further studies should certainly be made.

Chemical Properties.—The elastic tissue is composed of albuminoids which belong to the group of scleroproteins. In contrast to collagen, it is very resistant to the action of chemical agents (Satterthwaite). It is not soluble in acetic acid, potassium hydroxide or hot water. It is slowly digested by pepsin in an acid solution and by trypsin in an alkaline solution. The ease with which fibers are digested varies accord-

ing to the source from which the fibers are obtained (Marriott). When digested by trypsin in an alkaline medium, the fibers do not become thinner but break up into globules, which are held in position by an external sheath that may represent the substance with which fibers are "coated" or "impregnated." The fibers, as exemplified in certain pathologic states, have an affinity for calcium, iron and silver. Exposure of the skin of mice to roentgen irradiation, tar or arsenic causes an increase in the number of "elastic" fibrils (Bierich, 1922). Later Bierich and Rosenbloom used the term "resorcin fibres" to designate certain fibrils which they produced experimentally by depression of the

TABLE 1.—*Comparison of Collagen and Elastin (After Cohnheim)*

	Collagen	Elastin
Aminoacetic acid	19.25	25.75
Alanine	3.0	6.58
Valine	.....	1.0
Leucine	6.75	21.38
Aspartic acid	0.56	.....
Glutamic acid	14.0	0.76
Proline	7.7	1.74
Oxypyrrolidin carbonic acid	6.4	.....
Phenylalanine	0.4	3.89
Tyrosine	.....	0.34
Histidine	0.4	0.58
Lysine	5.6	2.46
Arginine	9.3	1.86
Ammonia	0.43	0.05

TABLE 2.—*Values Obtained by Chittenden and Hart in Analysis of Elastin*

Carbon	54.24
Hydrogen	7.27
Nitrogen	16.70
Sulfur	0.30
Oxygen	21.79
Ash	0.90

p_H of collagen to low values. They considered these to be products of hydrolysis of collagen and that they were related to absorption and release of silica compounds (Bierich, 1924).

Chemical analyses of the substance (elastin) which presumably represents the elastic tissue have been made in detail on ligamentum nuchae of oxen. Table 1 is taken from Cohnheim's textbook, "Chemie der Eiweisskörper," and table 2 includes the figures obtained by Chittenden and Hart. The comparison of elastin with collagen discloses certain important differences. An interpretation of these differences may aid in the explanation of some of the qualities of the elastica, namely, the acidity, the resistance to acids and the reducing power. These properties are those which were utilized in the development of differential staining methods. Elastic tissue contains only small amounts

of the basic diamino acids, and it is assumed that for this reason acids are less injurious to elastin than to collagen. The principal acid constituents of collagen are aminoacetic acid, glutamic acid, proline and oxypyrrolidin carbonic acid, while those of the elastica are aminoacetic acid, leucine, alanine and phenylalanine. These are less neutralized by basic amino acids, particularly lysine and arginine, than are the acids of collagen. Elastin also differs from collagen in that it has a strong reducing power. This has been attributed by Unna to its relatively high content of reducing amino acids.

Now that the physical properties and some chemical components of elastic tissue have been mentioned, the term "elasticity" may be defined, some of the laws which govern elasticity of solid bodies may be enumerated, and the rules which should be fulfilled to allow for elastic qualities in colloids may be considered. All this is done for the purpose of approaching the problem as to how well elastic tissue has been designed for the optimal performance of certain functions.

In the beginning, one must broaden one's views to include not only a perception of elasticity in the physical sense but also elasticity in the common sense. By physical definition, elasticity is the property in virtue of which bodies resume their original form or volume when the force which altered that form or volume ceases to act. By this definition the coefficient of elasticity of bone is much greater than that of elastic tissue. In the common conception of the term "greatness of elasticity" implies the property of great deformation when a force is applied, with almost prompt recovery of the original form or volume after the deforming force has ceased to act. Therefore "great elasticity," as a term of usage in this paper, implies qualities similar to those of a highly extensible rubber band.

Elasticity may be developed in bodies by pressure, traction, flexion or torsion. There is a limit of elasticity of solids beyond which they either break or are incapable of regaining their original form or volume. When the limit of elasticity has not been exceeded, the traction of rods and wires is subject to the following laws:

1. Rods and wires possess perfect elasticity; i. e., they assume their original length as soon as traction ceases.

2. For the same substance and the same diameter, the elongation is proportional to the force of traction and to the length.

3. For rods and wires of the same length and substance, but of different magnitude, the elongation is in inverse ratio to the squares of the diameters.

4. According to calculation and experiment, when bodies are lengthened by traction, their volume increases.

The laws of elasticity of flexure, which apply to bending and return to original form, and the laws of torsion need not be given in detail, but it is reasonable to believe that they may be applied to the elastic fiber.

In addition to the physical laws of elasticity, and inasmuch as the elastic fiber is generally believed to offer resistance to force, the rules of tenacity should be applicable. Tenacity is the resistance which bodies oppose to traction. It is directly proportional to the breaking weight and inversely proportional to the area of a transverse section of the wire. Tenacity diminishes with the duration of traction. A small force continuously applied will often break a wire which would not at once be broken by a larger weight. A cylinder has greater tenacity than a prism. The quantity of matter being the same, a hollow cylinder has greater tenacity than a solid one. In general for all bodies, the tenacity and elasticity are greater in the direction of the fibers than in a transverse direction. For most bodies, tenacity rapidly decreases as the temperature is increased.

There seems to be no valid reason for believing that elastic tissue does not obey these rules and laws. In this regard the wide differences in the texture and structural arrangement of elastic tissue may be emphasized. The application of these laws to the anatomic structure and distribution of the elastica becomes difficult because of the complications which arise when one attempts to analyze the part played by other elements, especially collagen and smooth muscle, with which the elastica is so intimately integrated. Nevertheless, a crude survey of the development, the localization and the variation in form and arrangement of elastic tissue shows how well this tissue has been designed to diffuse the forces of applied stresses, to allow for fluctuation in the shape of organs, to guard against the ill effects of excessive forces and to expedite the return of the deformed structure to its natural state. A complete dissertation on this subject could be given only after most extensive studies. Such studies have not been made.

To supplement the possible application of physical laws as outlined one may turn to an analysis of the factors which, according to Busse, govern the property of high elasticity in elastic colloids. First, there must be groups of atoms which form somewhat flexible fibrous units. Most requisite to this factor are long fibers. Of these, elastic tissue has an abundance, for, as has been seen, the continuous networks exhibit no free ends, and the elastic fibers may be traced for great distances without interruption. This is particularly true in the vascular system. Secondly, the fibrous unit of the elastic colloid must have weak or uniform cohesive (secondary valence) forces around them. From the roentgen ray spectograms it has been possible to assume that

the elastic fiber has weak secondary valence forces. Thus, in this respect, also, it is designed for high elasticity. Furthermore, on morphologic grounds it may be concluded that the fibers, as a rule, are so related to neighboring elements that they are enabled to stretch and retract with minimal hindrance when such movements are necessary. Thirdly, the highly elastic colloid must be composed of a three dimensional network which is formed by chemical combinations, secondary valence forces or mechanical entanglements. In elastic tissue the first means of interlocking the fibrous units, if present, is unknown. Roentgen ray analysis gives some reason for believing that there are weak secondary valence forces. The third means, which is of great importance, is exemplified by the fundamental structural arrangement of the continuous meshwork of elastic fibrils. Fourthly, colloids of great elasticity must have a means by which free energy is stored during the process of deformation, to be utilized in the performance of external work during the process of recovery to the original form or volume. It is theoretically plausible that the means by which the elastic fiber stores energy on deformation may be through a distortion of molecules, as evidenced by the assumption of doubly refractive qualities on stretching, with consequent accumulation of potential energy which is available for doing work. What part the reducing factors of certain components of the elastic fibril may play is a problem which remains unsolved. There is no reason to believe that this chemical characteristic is requisite for elasticity of the fiber. From the data given it may be inferred, therefore, that the elastic fiber fulfils so far as is allowed by the limitations of the available data those requirements by which bodies achieve high elasticity.

STAINING METHODS AND REACTIONS

It is not advisable to give detailed accounts of the various technics of staining. It seems more appropriate to outline the evolution of staining methods, to evaluate their usefulness, to describe normal and pathologic reactions and to present theoretic explanations for the affinity of elastic tissue for certain "specific" stains. Any assumptions which might arise concerning the functional integrity of the elastica based on arbitrary criteria of staining reactions should be tempered by the fact that optical properties are characteristic before the development of an affinity for specific stains. Furthermore, a survey of the staining reactions of elastica in pathologic states indicates that an abnormally intense or a weak reaction may not necessarily have the great significance that many authors have assumed. Neither are normal reactions exact criteria by which function may be measured.

The physical and chemical differences between elastic tissue and collagenous tissue are the means by which microscopists have been enabled to distinguish the two types of tissue and to perfect differential stains. The physical structure and especially the optical properties were utilized by the earliest histologists in recognition of elastica. In later years the chemical differences became more apparent. These gave origin to two terms, "elastin" and "collagen." The former was used to designate the chief substance of elastic tissue, and the latter, of fibrous connective tissue. It was discovered that elastin was more acid than collagen, that collagen swelled in acid solutions while elastin did not, and that elastin had certain strong reducing characteristics which were not possessed by collagen.

Virchow (1851) found that when tissues were treated with acetic acid the collagenous fibrils became swollen and thereby could be distinguished from elastic fibrils, which were unaffected. The swelling of collagen in acids and the inherent acid character of elastin were the observations on which early staining methods were founded. These methods consisted in the utilization of acids, such as nitric acid (Taenzer) and sulfuric acid (Manchot), as well as of basic dyes, such as fuchsin or safranin.

The reducing power of elastin was used in the further development of staining methods. It was discovered that elastic tissue when treated with potassium permanganate becomes dark brown and thereby is differentiated from collagen. Also, in contrast to collagen, elastin attracts and reduces the highly oxidized metallic acids, such as osmic acid and chromic acid. By this means the acidity of elastin may be increased so as to enhance its affinity for basic dyes. On the principle of the reducing power of elastin, the two most useful stains were developed. These are the orcein stain (Taenzer-Unna) and the resorcin-fuchsin stain (Weigert).

The theory of the Weigert stain as given by Unna (1928) is as follows: The acid elastin has a chemical affinity for basic fuchsin. Added to this is the affinity of the "reducing" elastin for an oxidizing agent, ferric chloride. This reaction is enhanced by treatment with the bivalent phenol, resorcinol, which is a strong reducing agent. The use of resorcinol is not essential, because fuchsin and ferric chloride will stain elastin in a specific manner. Ferric chloride may be replaced in this reaction by other oxidizing agents, such as ammonium persulfate, chromic acid and potassium dichromate. The advantage in using ferric chloride is that the reduction of this chloride by elastin leads to the formation of hydrochloric acid, an acid which causes collagenous fibrils to swell, thereby inhibiting their affinity for the basic dye fuchsin.

Although the Weigert stain is very satisfactory, many authors have given preference to the orcein stain (Taenzer-Unna) because other

differential stains can be used at the same time, because the detail of delicate fibrils is somewhat better and because the Weigert stain is useless for the differentiation of elastin and elacin. Orcein is a pure acid dye that combines with the acid substance elastin in an oxypolar manner. This combination is brought about through the capacity of elastin to reduce orcein. The addition of nitric acid is necessary because of its effect on the collagen. Numerous variations of the orcein and resorcinol-fuchsin stains have been developed. In general, the basic principles are the same. No attempt will be made to describe the numerous modifications, but certainly the Verhoeff stain is very reliable, and many believe that there are advantages in its use.

Elsewhere in this review it has been shown how the normal "mature" staining reactions of elastic tissue gradually developed, phylogenetically in the animal series, ontogenetically in man and culturally in studies in vitro. So far as I am aware, no experimental evidence has been brought forward which will explain the increasing intensity of the so-called specific staining reaction. Any information in this regard should shed some light on the means by which elastic tissue attains certain singular capacities. It must be kept in mind that in some instances the colloid in the thyroid gland, mucin, collagen and basement membranes may elect the specific stains. Otherwise, for practical purposes the orcein and resorcinol-fuchsin stains demonstrate the normal elastic tissue in a satisfactory manner.

The alterations which occur in the elastica may be distinguished not only by atypical reactions to the specific stains but also by various structural changes. It may be emphasized here that a change in the physical properties or in the chemical structure of the fiber does not necessarily imply that there is a comparable change in the uniformity or intensity of staining. Neither does a change in the staining reaction signify in all instances a parallel change in the functional ability or in the structural integrity of the fiber. Therein lies the weakness of many dogmatic interpretations of histologic appearances.

Most authors have expressed the belief that the first stage of atrophy is a partial or complete loss of elective staining qualities. The first fibers to be involved are the delicate fibrils. At the same time the larger fibers become separated into segments, often with alternation of stained and unstained parts. Following this they lose their specific staining qualities, diminish in thickness and eventually disappear. Krösing and Passarge contended that this was true because, as they showed, in tuberculous areas where the elastica had largely disappeared treatment of the tissues with potassium hydroxide enabled them to visualize elastic fibers that previously could not be distinguished. Unna (1896) noted that some of the fibers of senile skin were stained weakly

with orcein. These had an affinity for basic aniline dyes. He used the term "elacin" for the changed substance of these fibers to distinguish it from elastin of normal fibers. Also the senile skin contained a material which had the structure of collagen and the staining qualities of elastin. He called this material "kollastin." There also was an element which had the staining qualities of elacin and the structural arrangement of bundles of collagen. He named this "kollacin." The significance of these elements is unknown. In general, modern authors have accepted the descriptive terms "elastin" and "elacin." The various types of fibers may be found in numerous pathologic states, and due consideration will be given them elsewhere.

The elastic fiber may give evidence of the effect of disease processes in many other ways. It is common for networks to become disrupted and to form skeins, tangles and more or less homogeneous masses. The fibrils may swell, either diffusely or in such a way that the varicose thickenings become so spaced as to resemble a rosary. They may fracture, usually transversely, and curl at their ends. Rarely, they are split longitudinally. They tend to retract spirally and flex to form symmetric arcs. They may break up into a series of refractile elastic granules, reminiscent of the embryonic prefibrillar granule stage which has been described by many authors. These granules may occur in strands, gather in clusters or fuse to form homogeneous masses (Schmidt). Vacuoles which contain fat may be found in the fibrils (Jores, 1902). Therefore, let it suffice for the present to say that the elastic fiber may undergo slight or profound morphologic changes with or without loss of the specific staining reactions of its component parts and that the staining reactions may vary with or without changes of a morphologic nature.

PHYSIOLOGY

The methods which have been applied to the investigation of the function of elastic tissue have given results which as a rule will not bear close scrutiny. In the first place, authors have couched their results either in generalities or in terms of resistance, retraction, extensibility or coefficient of elasticity. In the second place, elastic tissue that was entirely devoid of other conflicting tissue elements has not been used in the experiments. As a result, confusing and contradictory findings have burdened the literature.

Direct observation of the isolated elastic fiber has led to general acceptance (Maximow; Sobotta) of the supposition that the fiber is highly extensible and has inherent ability to return promptly to its natural size and shape. Despite the accepted belief that elastic tissue is of great importance because of its elastic qualities, several authors, especially Sternberg and MacLeod, have contended that the chief func-

tion of the elastic network is one of support—a restraining structure designed to prevent overdistention of tissues.

Much of the dissension has arisen because of opinions which have been founded on the interchangeability of staining reactions of elastic tissue, collagen and basement membranes. This is dependent on a concept of a theoretic substance, elastin. This concept, as has been stated, embodies the belief that fibers may be impregnated with this substance and that they thereby assume elective staining qualities. The hypothetic impregnation and disimpregnation of collagenous and elastic fibers with hypothetic preformed materials, elastin and collagen, have given rise to such terms as "elastin," "elacin," "collastin" and "collacin." The elastic properties of elacin, collacin and collastin are unknown, but it reasonably may be assumed that the staining reaction is not acceptable as an absolute measure of their functional capacity. Basement membranes, which often stain similarly to elastic tissue, are not necessarily endowed by this staining property alone with the same functional capacity as elastic tissue. Nevertheless, these membranes, as well as collagen and reticulum, possess certain elastic qualities. How these compare with the elastic qualities of elastic tissue remains a question that has not been answered.

Triepel contended that the elasticity coefficient when distending forces were used was greater for collagen than for elastic tissue. When shearing forces were applied, the elasticity coefficient for elastic tissue was greater than that for collagen. By no means do these findings aid one in a solution of problems involving the question of great extensibility and retractile power, for, as is well known, bone has a higher elasticity coefficient than either collagen or elastic tissue.

Certain studies have been made on the elasticity of organs. An organ or tissue containing elastic fibers does not attribute its elasticity to elastic fibers alone. If an organ or tissue is so constructed as to allow for great deformation, a fairly prompt recovery of that organ to its natural size implies that most of its mobile component parts are capable of withstanding great deformation, subsequently returning to normal form. The ability of the organ to resume its natural size depends largely on its structural units. Abnormality of any one of the important units may alter the return of the organ to its natural size and shape by interfering with or enhancing the function of the active elements. The most important active elements are of muscular nature. Cardiac and voluntary striated muscle may be largely disregarded. Smooth, or involuntary, muscle, which is so widely distributed, plays a major role by virtue of its extensibility and its contractile power. Intimately associated with this tissue in so many situations is elastic tissue. Indeed, this association is often so pronounced that the term "myoelastic tissue" has been utilized to designate the combination.

Furthermore, in certain situations the elastica occupies positions where one might well expect smooth muscle. The integration must signify something more than simple support of smooth muscle by the elastica. In the first place, the continuous elastic network is so placed as to aid in the dissemination of stresses directed at isolated points. Secondly, the network is ably designed for coordination of rhythmic movements of separate units. Thirdly, it aids in the conservation of energy through partial maintenance of tone during relaxation of the muscular elements. Fourthly, because of its great tenacity and the continuous character of the membranes and networks, it serves as a bulwark against the possible injury of excessive forces. Fifthly, because of the relatively great extensibility and inherent power of prompt retractility of the elastic fiber, it aids materially in the return of a tissue or organ to the natural form after a deforming force has been removed.

The roles of the remaining primary tissue elements, such as collagen, reticulum, mucin, cartilage, bone and cell structures, are indispensable. All must play some part in the return of distorted organs or tissues to natural forms, but because of basic physical properties or because of anatomic arrangement they become of secondary value in the general scheme. Among these, collagen is of greatest importance because of its almost constant neighborly association with elastic tissue. Thus the physiologic or the pathologic state of one is almost always reflected by functional or structural changes in the other. With this in mind and with the knowledge that pathologic processes usually affect both elements simultaneously, the interpretation of changes in elasticity of tissues must be guarded.

In relation to arteries it has been said that the amount of elastica roughly parallels physiologically the pressure within the artery (Maximow and Bloom). Sternberg stated that the number of fibers in a given tissue is not an absolute index of the degree of elasticity of that tissue. As an example, he cited arteries of the muscular and of the elastic type. Although there is little difference in the elasticity of these two types of vessels, there is great difference in the amount of elastica.

The most illuminating and careful researches dealing with the physiologic importance of elastic tissue have been conducted by the school of English physiologists. Their studies have been concerned chiefly with the function of the elastica of blood vessels. I have drawn liberally from the excellent presentation of this work as given by Bramwell.

It has been learned that arteries may be divided on the basis of their constituent elements into vessels of the elastic type, vessels of the muscular type and vessels of a transitional structure. Typical elastic arteries are the aorta, the subclavian artery and the carotid artery. The

muscular arteries may be exemplified by such vessels as the radial and the lingual. The axillary and the common iliac arteries may serve as examples of the transitional forms. It has been shown that under normal conditions the smaller arteries, such as the radial, are less elastic than the larger vessels, especially those of the "elastic" type (Bazett and Dreyer; Fulton and McSwiney). It has been learned that there are certain diseases which affect the vascular system in such a manner as to reduce the elasticity of vessels. The vessels which are affected in these conditions and the degree of their functional impairment are such as to exert an important influence on the circulation of the blood. For this reason this subject will be given further consideration in the following paragraphs.

The efficiency of arteries as judged from one point of view must depend principally on their elastic qualities. During the initial phase of ventricular systole the cardiac output is accommodated largely by distention of the aorta. The rapid stretching of the aorta and other arteries allows for a storage of potential energy in the vascular wall. This energy is utilized during the period of retraction in promoting the forward movement of the blood column. The greater the elasticity of arteries the lower will be the pressure required to produce a given increase in volume for the accommodation of the cardiac output. Since the energy expended by the ventricle in systole is proportional to the pressure developed, the effort of the ventricle will vary inversely with the extensibility of the arterial walls. Similarly, during the phase of arterial retraction the more elastic the arteries the smaller is the fall of pressure for a given decrease in their volume. In other words, the more elastic the arterial walls the lower is the pulse pressure and the more uniform the blood flow through the capillaries. The conclusion may be reached that the greater the alteration in volume of arteries in response to a given alteration in pressure the more efficient is the arterial mechanism.

Since the velocity with which the pulse wave is transmitted along arteries depends principally on the elasticity of the arterial walls, direct observations on the velocity of the pulse wave have been used as a basis for the calculation of the mean extensibility of vessels in absolute units (Bramwell and Hill). Bramwell, Hill and McSwiney showed that in normal healthy persons the velocity of the pulse wave increased with age. They found that the elasticity of arteries was halved between 10 and 60 years of age. Comparable results were obtained from a study of arteries removed from subjects post mortem. This variation in arterial elasticity may be attributed principally to structural changes in the arterial wall even though, as has been stated previously, these changes are not always demonstrable by histologic study.

In a study of isolated human arteries it was found that at varying internal pressures, as the diastolic pressure was increased a disproportionately large superimposed pressure was necessary to produce the same percental increase in the volume of the vessel. The tension with which the artery was stretched longitudinally did not affect the circular elasticity to any appreciable degree. The results were explained by the hypothesis that the arterial wall consists of a series of different elastic systems. At very low pressures only the most distensible of these are stretched. As the pressure rises, less and less distensible systems are called into play. At low pressures the artery exhibits a high degree of elasticity. At higher pressures the pulse velocities become a linear function of pressure since the vessel becomes relatively nonelastic.

Wilens made a careful study of the postmortem elasticity of the aorta. He found that restraint of the intima often disturbed measurements of elasticity, and he eliminated this source of error by stripping the intima from the media. The results of his measurements show that postmortem elasticity is almost exclusively a function of age and is relatively constant in any given age group. The mobile parts of the aorta retain more elasticity than the fixed parts.

There is little relationship between intimal lesions and loss of elasticity of the regional media. The elasticity is approximately equal in longitudinal and transverse directions of all segments of the aortic wall.

It is interesting that in certain wasting diseases the elastic element of the arterial wall appears to lose much of its efficiency (Bramwell, Downing and Hill). This finding is comparable to similar results obtained from a study of the elasticity of the skin by Schade (1912). Furthermore, hypoextensible and hyperextensible arteries have been found in apparently normal subjects. Such individual variations also occur in the elasticity of the skin and possibly in that of the lungs of normal people.

The elasticity of the lungs, which are rich in elastic tissue, is of great importance in respiratory mechanics. This elasticity does not change materially for several days after death (Bönniger, 1908). The great increase in the volume of lungs during inspiration takes place mainly through distention of alveolar ducts and to a lesser degree through distention of the bronchi and bronchioles rather than through that of alveoli (Maximow and Bloom). These parts of the lungs which have greatest fluctuation in size during normal respiration have the richest supply of elastic tissue. In a previous part of this article it has been stated that the full development of the pulmonary elastica is not attained until the lungs have been used for respiration. According to Cloetta, the expiratory movement is a purely elastic phenomenon, and the collapse of the lung in pneumothorax is due more to retraction of the elastica, which is under constant tension, than to closure of the

small bronchi. On the basis of experimental studies Cloetta concluded that for expansion during inspiration the lung possesses ideal elasticity. Bönniger found that the lungs of children were more elastic, in the commonly accepted sense, than those of adults. This roughly parallels the conception of the elasticity of the skin and arteries.

The skin because of its availability has received more study, and an effort has been made to relate the changes in the elasticity of the skin to various local and systemic pathologic processes (Bönniger, 1904-1905). An excellent discussion of methods of measuring elasticity is given by Reuterwall. The resistance of the skin to palpation has served as a crude index of elasticity, but Schade devised an instrument by which the relaxation time of the skin could be measured. This mechanical device was called an elastometer, and the application was known as elastometry. By this method of study Schade found that a disturbance in elasticity was in general an early symptom of organic disease. He obtained low values in diabetic acidosis, myxedema, pernicious anemia and septic fevers. In septic fevers the values were 12 to 26 per cent below normal. It may be interesting to speculate on the effect of changes in elasticity in other organs, if one assumes that they may parallel cutaneous changes. Such a change has been suggested as affording a partial explanation of the soft splenic tumor which often accompanies infection in the adult (Lubarsch). Schade (1912) found that elasticity of the skin diminished with increasing age and was least in old persons. In edema of the skin elasticity was reduced even before edema was palpable. The ratio between the degree of edema and the loss of elasticity was not constant, but the average reductions varied from 20 to 50 per cent. In inflammatory edema the diminution in elasticity was of similar degree, and Schade (1912) believed that comparable losses were probably present in lungs affected by pneumonia.

The effect of fatigue on the elasticity of connective tissues has been studied. Schade (1921) found that a sleepless night reduced the elasticity of the skin about 15 per cent. Katzenstein measured the amount of fluid which was necessary to fill knee joint capsules of rabbits before and after exercise of the joint. He demonstrated that prolonged active and passive movements of the joint were followed by a substantial increase in the capacity of the joint capsule. It was assumed that this was the result of relaxation in the fibers of the joint capsule.

(To Be Concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—T. Wingate Todd, professor of anatomy in Western Reserve University and director of the Brush Foundation, Cleveland, died Dec. 28, 1938, at the age of 53.

A. F. Bernard Shaw has been appointed to the joint post of professor of pathology in the University of Durham and pathologist to the Royal Victoria Infirmary, Durham, England, in succession to Stuart McDonald.

Karl Sudhoff, the medical historian, died Oct. 8, 1938, in his eighty-fifth year.

According to the *Lancet*, Phillipp Schneider is the new director of the Institute of Forensic Medicine in Vienna.

Ernest E. Tyzzer, professor of comparative pathology in Harvard Medical School, has been appointed professor of tropical medicine also, succeeding Richard P. Strong, retired.

Henry Pinkerton, assistant professor of pathology at Harvard Medical School, has been appointed professor of pathology in St. Louis University, St. Louis.

In the Pasteur Institute, Paris, Harry Plotz has been placed in charge of virus research.

G. B. Magrath, professor emeritus of legal medicine in Harvard Medical School, died Dec. 10, 1938, at the age of 68 years.

Harvard Symposium on Viruses.—The Harvard School of Public Health offers a course of lectures, clinics and demonstrations on the virus and rickettsial diseases, with special emphasis on their significance for public health, June 12 to 17, 1939. Lectures on the etiology, epidemiology and methods of control of these diseases, by members of the faculties and by former students of the Harvard School of Public Health and of the Harvard Medical School, will occupy five mornings. Special clinics and demonstrations will be given each afternoon. On the last morning, a panel discussion will be held on the three main topics presented in the symposium. The fee for the course will be $25. Enrolment should be arranged before June 1, as facilities for many of the clinics and demonstrations are limited. The lectures will be published later in a single volume, which will be sent to each registrant for the course. For further information, write to the Secretary of the Harvard School of Public Health, 55 Shattuck Street, Boston, Mass.

Society News.—The eighteenth annual meeting of the American Society of Clinical Pathologists will be held in St. Louis, May 12, 13 and 14, 1939. The Hotel DeSoto will be the official headquarters.

The American College of Physicians will hold its twenty-third annual session in New Orleans, March 27 to 31, 1939.

Celebrations.—The sixtieth birthday of Howard T. Karsner and his twenty-fifth year as professor of pathology in Western Reserve University were celebrated Jan. 6, 1939, when a portrait of Dr. Karsner was presented to him.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Dermatoses Due to Vitamin A Deficiency. J. B. Youmans and M. B. Corlette, Am. J. M. Sc. **195**:644, 1938.

A dermatosis due to deficiency of vitamin A is described, with the histologic studies. Some of the patients presented the dry horny papular lesion described by Frazier and Hu; others presented an acne-like lesion that differed in some respects from those previously described. The histologic picture and the response to treatment with vitamin A were similar in the two types of eruption.

The relation of these changes in the skin to the other manifestations of avitaminosis A is of considerable importance. Xerophthalmia and hemeralopia were present in most of the cases reported by Loewenthal and in many of those reported by Frazier and Hu. Cornification of the epithelium of the conjunctiva was observed by the latter in patients with less severe lesions of the eye. Nicholls' patients had night blindness. In none of the patients whom Youmans and Corlette studied were there changes in the epithelium of the eye, and only occasionally did one give a history of mild night blindness, and then the history was questionable. Studies with a visual photometer demonstrated in some of the subjects occasional mild night blindness. Blackfan and Wolbach and others showed that the manifestations of a lack of vitamin A are evident in the epithelium of many tissues and organs and that these changes vary in respect to the order of their appearance and their severity. Although the changes in the eye and in the vision have been thought to be the earliest reliable clinical manifestations of avitaminosis A, the observations of Youmans and Corlette, as well as those of Frazier and Hu, suggest that in some instances the cutaneous lesions may be among the first clinical evidences of the deficiency, appearing before demonstrable changes in the epithelium of the eye and before more than a mild night blindness, detectable only by a photometer, is present. If so, these changes may constitute one of the earliest signs of vitamin A deficiency, fortunately easily recognizable.

From Authors' Summary.

Renal Insufficiency from Blood Transfusion. E. L. DeGowin, E. D. Warner and W. L. Randall, Arch. Int. Med. **61**:609, 1938.

The transfusion of canine hemoglobin into dogs when the urine is acid results in death from renal insufficiency. This does not occur when the urine is alkaline at the time of the transfusion. The anatomic picture of obstruction of the renal tubules by hemoglobin pigment sufficient to be the chief cause of the renal insufficiency is observed in most dogs under the experimental conditions outlined. A nephrotoxic process often operates and may cause renal insufficiency independently. The deposition of hemoglobin pigment as hemosiderin in the renal tubules and in the reticuloendothelial system apparently does not contribute to the development of renal insufficiency. An anatomic study of the kidneys of 9 human beings who died of renal insufficiency after hemolysis revealed the two independent mechanisms seen in dogs, the obstruction with pigment and the necrosis. In occasional human beings the precipitation of hemoglobin pigment in the tubules is extensive and may be a cause of renal insufficiency. This complication could probably be prevented by alkalinizing the urine prior to the transfusion. In the majority of human beings showing renal insufficiency after hemolysis the condition is probably caused by some nephrotoxic substance which causes degeneration of tubular epithelium and interstitial edema.

From Authors' Summary.

CHANGES IN THE BRAIN IN PLEXECTOMIZED DOGS, WITH COMMENTS ON THE CEREBROSPINAL FLUID. G. B. HASSIN, E. OLDBERG and M. TINSLEY, Arch. Neurol. & Psychiat. **38**:1225, 1937.

The authors tried to determine the effect of removal of the choroid plexus on the size of the cerebral ventricles and the changes that take place in the brain in such experiments. In some animals the sylvian aqueduct was blocked without removal of the choroid plexus; in others the choroid plexus was either removed from one lateral ventricle or left in the latter after closure of the ipsilateral foramen of Monro with a piece of fascia and muscle; in another group both choroid plexuses were removed after blocking both the foramina of Monro. Plexectomized brains invariably exhibited connective tissue scar formation, degenerative, reactive and inflammatory phenomena, ependymitis and subependymitis, which may affect the size of the ventricles. It was not possible to remove the choroid plexuses in toto, for small fragments of them could be demonstrated under the microscope. The results of plexectomy were by no means uniform, as in some cases a plexectomized ventricle with the foramen of Monro blocked appeared to be of the same size as the opposite ventricle or smaller or even larger. Like pathologic observations on hydrocephalus in man, those following experimental plexectomy force one to the conclusion that the cerebrospinal fluid accumulates in the ventricles but is derived not from the choroid plexus but from the tissue fluids of the brain, which are drained by the ventricles and subarachnoid space. It is not an organ of secretion but of excretion, eliminating from the spinal fluid substances which are harmful to the nervous system and which render the fluid more absorbable.

GEORGE B. HASSIN.

BLEEDING TENDENCY AND PROTHROMBIN DEFICIENCY. H. P. SMITH and others, J. Exper. Med. **67**:911, 1938.

In dogs with biliary fistula the plasma prothrombin falls eventually to low levels, and bleeding commonly occurs. An important causative factor in this fall is the faulty absorption of vitamin K from the intestine in these animals. Feeding bile permits absorption of the traces of this vitamin normally present in mixed diets, and, as a result, a slow rise in prothrombin is observed. If a standard diet is supplemented with large amounts of vitamin K concentrate, the rise is rapid, provided bile or bile salt is supplied to aid in the absorption. Variations in the rate of depletion of prothrombin in dogs which have biliary fistula and which are kept on a constant diet indicate the existence of additional factors which require further study. The experience of Smith and his co-workers indicates that vitamin A and vitamin D supplements do not correct the prothrombin deficiency in animals with biliary fistula.

FROM AUTHORS' SUMMARY.

PERIPHERAL BLOOD PHENOMENA AND DIFFERENTIAL RESPONSE OF BONE MARROW AND LYMPH NODES TO HYPERPYREXIA. C. A. DOAN, Radiology **30**:382, 1938.

The hemopoietic response to "fever" is rather constant, and the majority of the cells making up the postfebrile leukocytosis are polymorphonuclear neutrophils, newly delivered by the bone marrow, as shown by their youth. This part of the reaction may be nonspecific and is by no means necessarily the most important from the standpoint of the fundamental defenses of the body. There is destruction of lymphocytes during hyperpyrexia, as attested by the studies of lymph nodes cited and by the return of very young cells to the circulation after prolonged lymphopenia. There is probably in the human patient some destruction or redistribution of monocytes, as is shown by the delayed monocytosis, made up primarily of younger forms. The hemograms made after inoculations of malarial parasites and of typhoid vaccine differ from those observed during fever induced by physical methods in the marked leukopenia during the chill, in the temporary disappearance of the monocytes from the circulation following typhoid and in the marked stimulation of the monocytes in malaria and their moderate stimulation following

inoculation of typhoid vaccine. The shift to the left in the neutrophilic granulocytes in malaria is outstanding, and the appearance of clasmatocytes in the peripheral blood has been observed with no other type of experimental fever. It has been suggested by Breutsch that the profound stimulation of phagocytic clasmatocytes observed in malaria as the result of the destruction of red blood cells by plasmodia provides an important cellular defense weapon in the treatment of syphilis of the central nervous system, which is not available when other fever-producing methods are employed. Cunningham has emphasized the importance of clasmatocytes in the control of experimental syphilis in rabbits. While it is true that biopsies of the sternal marrow in the human patient cited, and the bone marrow of rabbits studied post mortem following hyperthermic fever therapy did not show an increase in clasmatocytes, there was a tremendous increase in these phagocytic cells elsewhere in the tissues, more especially in the lymph nodes, spleen and liver. To that extent, at least, hyperthermia, produced by physical means, not only provides the thermal factor of importance for the inactivation of Spirochaeta pallida and the gonococcus but, as now demonstrated, exerts a profound effect on the cellular equilibriums of the body—in the directions which, it is believed by the author, are the most effective in mobilizing the defense forces of the body against these organisms. In short, hyperpyrexia acts as a two-edged sword cutting both ways in its role as "assistant extraordinary" to the humoral defense mechanisms of the body.

FROM AUTHOR'S SUMMARY.

THE EARLY LESIONS OF EXPERIMENTAL ENDOCARDITIS. W. DIETRICH, Virchows Arch. f. path. Anat. **299**:285, 1937.

On the theory that sensitization causes an increase in the reactivity and resorptivity of all the tissues, rabbits were sensitized by repeated injections of horse serum, caseosan (a product which is essentially a 5 per cent solution of casein), colon bacillus vaccine and histamine. After sensitization suspensions of living colon bacilli or of staphylococci were injected intravenously; these injections were repeated in the course of four to five days. At intervals of seven to seventeen days later the animals were killed and the cardiac valves examined microscopically. The earliest changes noted were swelling of the endothelium and varying degrees of edema of the subendothelial tissue, together with moderate cellular proliferation of this layer. Bacteria in small numbers were found in the endothelium and in phagocytic histiocytes of the subendothelial tissue. A thin layer of fibrin was often deposited on the surface of the endothelium; bacteria were deposited in this fibrin layer and multiplied in it. Horse serum and caseosan evoked a more marked reaction than the other sensitizing substances used. When living colon bacilli were used for the provocative injection, the slight, early changes described resulted. Living staphylococci led to acute ulcerative endocarditis.

O. T. SCHULTZ.

NARCOSIS AND HYPERERGIC INFLAMMATION. W. EICKHOFF, Virchows Arch. f. path. Anat. **299**:300, 1937.

To determine whether narcosis, which prevents the onset of anaphylactic shock in sensitized animals, also affects the hyperergic vascular inflammatory reaction, rabbits and guinea pigs were sensitized with swine serum. Under deep and prolonged narcosis the animals received one or more large intravenous or intracardiac provocative doses of the antigen. The prevention of immediate symptoms of shock was found to depend on the depth and duration of the narcosis. Rabbits anesthetized by the inhalation of ether escaped immediate shock but lived at the most ten hours. The lungs of such animals were deeply congested and revealed early pneumonic changes, ascribed to the cooling and irritant action of the anesthetic. Animals narcotized by the intravenous injection of ethyl carbamate (urethane) did not show anaphylactic shock; they recovered from the narcosis and appeared well. Such animals were killed at intervals of eight, fourteen and

twenty-one days after injection of the provocative dose of the antigen. Animals that lived fourteen days or longer revealed the perivascular histiocytic and leukocytic inflammatory reaction, similar to that of periarteritis nodosa, considered to be the characteristic hyperergic effect on the vessels. Ethyl carbamate narcosis delayed but did not completely prevent hyperergic inflammatory reaction. Vascular reaction was most marked in the heart, liver and kidneys. Changes in the hydrogen ion concentration of the blood and in the vascular regulatory mechanism of the autonomic nervous system are believed to be factors in the effects of narcosis.

O. T. SCHULTZ.

Pathologic Anatomy

ABNORMAL DISTRIBUTION OF THE SUPERFICIAL MUSCLE BUNDLES IN THE HUMAN HEART. J. S. ROBB and R. C. ROBB, Am. Heart J. **15**:597, 1938.

Fifty human hearts have been dissected to demonstrate the ventricular muscle bands. The superficial and deep sinospiral and bulbospiral muscles were present in all hearts. The authors know of no report in which these muscles were stated to be absent. The surface pattern of these muscles is variable, especially at the lower part of the anterior surface of the right ventricle, in the right ventricle near the conus, along the trabeculated area and at the anterior horn of the left ventricle. The angle at which the superficial sinospiral muscle fibers pass from the anterior horn to the right base varies considerably. In the small heart the fibers have an oblique course, tending to approach a vertical course from apex to base. In the hypertrophied heart these fibers have an almost horizontal course. The masses vary considerably. The right portion of the deep sinospiral muscle is differentially hypertrophied in mitral disease or in any other disease characterized by increased resistance to the flow of the pulmonary blood. The deep bulbospiral muscle is similarly hypertrophied in hypertension and aortic stenosis. If the work of the heart is much increased, the left portion of the deep sinospiral muscle may also hypertrophy.

The surface portions of the two superficial muscles do not have a measurable variation in thickness. When intraventricular pressure is increased, the papillary portions of these superficial muscles hypertrophy. Conversely, in a heart in which the mitral valve has a "buttonhole" opening and calcified leaves the papillary portions of these muscles atrophy. If the surface muscles are variable in distribution, or if they are deficient, apparent discrepancies may occur when one is localizing points of initial negativity, the origin of premature beats and other phenomena. For surface localization of electrical phenomena accurate sketches of the surface distribution of the cleaned muscle should be provided.

FROM AUTHORS' SUMMARY.

THE BONE AND CARTILAGE LESIONS OF PROTRACTED MODERATE SCURVY. A. W. HAM and H. C. ELLIOTT, Am. J. Path. **14**:323, 1938.

Protracted moderate scurvy was produced in young guinea pigs by feeding them diets containing less than adequate amounts of vitamin C. It was shown that longitudinal growth of bone continued under this regimen only because most of the limited amount of new tissue which formed was in the peripheral part of the epiphysial plate and in the ring of the diaphysis adjacent to the periphery of the plate. This resulted in weakness of the shaft in this location and nonsupport of the epiphysial plate. The chief manifestation of scurvy in the epiphysis was found to be a diminution in the amount of the bone supporting the articular cartilage. It was pointed out that in the adult whose growth is over the effects of scurvy would become apparent in sites where continual replacement of tissue occurs to compensate for wear and tear. It was suggested that certain features of osteoarthritis (the poorly maintained articular cartilages, the generalized diminution of the amount of bone in the skeleton and the osteophytes) would be

not unlikely effects of a long-continued moderate deficiency of vitamin C in the adult. Lastly the theory which postulates that vitamin C controls the jelling of intercellular substances was discussed in the light of others' findings and those of the authors, and no support was found for this theory.

FROM AUTHORS' SUMMARY.

NATURE OF THE "SILVER CELLS" IN MULTIPLE SCLEROSIS AND OTHER DISEASES. N. BLACKMAN and T. J. PUTNAM, Arch. Neurol. & Psychiat. **39**:54, 1938.

"Silver cells" were described by Steiner, in observations on multiple sclerosis, as small round bodies the size of a lymphocyte. They invariably contained argentophilic small granules of precipitated silver, which Steiner assumed to be phagocytosed spirochetes. Blackman and Putnam found these cells in lesions of the blood vessels of the brain (arteriosclerosis, trauma and hemorrhage) in which the possibility of local phagocytosis of micro-organisms could be excluded. By using additional staining methods on adjacent frozen sections from the same block the authors deduced that the silver cells are of glial origin, that the particles are not necessarily spirochetes and that they are probably of hematogenous origin (calcium, iron).

GEORGE B. HASSIN.

TISSUE SPACES OF THE KIDNEY. F. FUCHS and H. POPPER, Virchows Arch. f. path. Anat. **299**:203, 1937.

The demonstration of tissue spaces between the capillaries and the parenchyma of certain organs has introduced the concept of serous inflammation, in which serum escapes into the pericapillary space and may lead to disruption of the capillary, on the one hand, and of the parenchyma, on the other. Such a process in the liver has been termed serous hepatitis and has been described in an article previously abstracted. The present authors concerned themselves with the demonstration of tissue spaces in the normal kidney. The rabbit's kidney and the human kidney, obtained at necropsy, were used. India ink diluted with physiologic solution of sodium chloride (1:4) was injected into the pelvis under moderate pressure by means of a cannula in the ureter. The injected fluid entered the perivenous spaces of the pelvis and passed distally through the parenchyma of the organ. This procedure was sometimes combined with injection of colored gelatin into the arteries. A space filled with the ink surrounds the capillary and separates the latter from the tubule.

O. T. SCHULTZ.

INTERRELATIONSHIP BETWEEN THE LIVER AND THE BRAIN. V. NICOLAJEV, Virchows Arch. f. path. Anat. **299**:309, 1937.

The association of changes in the brain and liver in Wilson's disease led to a histologic study of alterations in the brain and liver in various diseases of the latter organ in man and in animals subjected to a variety of hepatic poisons. The results of this study were recently published in Lettish. The present article is an eight page summary of the previously published observations. Degeneration and disintegration of the hepatic parenchyma lead to the formation of "hepatogenic toxins" that have a deleterious effect on the brain, resulting in functional insufficiency of the glia and in degenerative changes, or a status spongiosus, of the glia. The degenerative changes are the result of alterations in the circulation. Such an effect on the brain results from a variety of pathologic processes in the liver. The changes in the central nervous system in Wilson's disease are characteristic, since they develop on the basis of a congenital anomaly of metabolism. Icterus is not a pathogenic factor in the cerebral changes observed.

O. T. SCHULTZ.

FIBRINOID DEGENERATION OF CONNECTIVE TISSUE FOLLOWING A SINGLE INJECTION OF PROTEIN. U. GRAFF, Virchows Arch. f. path. Anat. **299**:339, 1937.

Graff criticizes the tendency so evident in the current literature to term hyperergic the inflammatory reaction observed in a constantly increasing variety and number of human diseases on the basis of similarity of alterations produced in the specifically sensitized animal. He attempts to show experimentally that similar changes and especially the fibrinoid degeneration of connective tissue that many hold to be characteristic of hyperergic inflammation can be produced by a single injection of foreign protein and therefore are not evidence of hyperergic reaction. Rabbits and cats were used, but chiefly rabbits, in the relatively small series of experiments reported. Admitting that a change in the reactivity of the vessels is necessary, the ear of the rabbit was warmed in water at 40 C. before injection of the protein, or the latter was preceded by an injection of allylforminate, which renders the vessels more permeable. The animals were killed at intervals of from two hours to fourteen days after the injection of protein, and the tissues were examined histologically. The protein chiefly used was swine serum. Graff describes fibrinoid degeneration and cellular infiltration that he claims are similar to those seen in hyperergic inflammation. He concludes that his results establish the correctness of his thesis that not every so-called hyperergic inflammation is the result of sensitization. Graff's communication is followed by a note by Roessle (p. 359), who had the opportunity of examining Graff's preparations. Roesslè severely criticizes Graff's observations and interpretations. He denies that there is identity or even great similarity between the changes produced by Graff and those which competent pathologists consider characteristic of experimental hyperergic inflammation. What Graff terms fibrinoid degeneration is not such. Unusually large amounts of swine serum were injected. This may have had a primary toxic action, or the slow degradation of the serum may actually have sensitized the animals. In Graff's experiments from three to seven days were required for the development of the changes described. This, according to Roessle, is in striking contrast to the rapidity with which allergic hyperergic inflammation develops in the experimental animal.

O. T. SCHULTZ.

PARTICIPATION OF THE LYMPHATICS IN PATHOLOGIC ALTERATIONS OF THE SPLEEN. E. JAEGER, Virchows Arch. f. path. Anat. **299**:552, 1937.

In conditions leading to stasis in the portal system the quantity of lymph drained from the spleen is increased over the normal. The hilar lymph nodes of the spleen enlarge and take on a reddish brown color, which has led to the belief that they become transformed into hemolymph nodes and take over part of the function of the spleen. Although it is difficult or impossible to demonstrate deep lymphatic channels in the spleen by injection methods, dilated endothelial-lined spaces are evident in the periadventitial tissue of the arteries in conditions of stasis. By means of serial section reconstructions Jaeger was able to show that these spaces are part of a network of lymphatic vessels which begin in the malpighian bodies and run in the periarterial tissues to the hilus of the spleen. In venous stasis of the spleen erythrocytes are forced into the lymphatic channels. In the malpighian bodies the deposition of iron pigment leads to the formation of the Gandy-Gamna iron and calcium incrustation bodies. Deposition of the pigment in the hilar nodes transforms these into pseudohemolymph nodes. Periarterial fibrosis of the spleen results from inflammatory reaction on the part of the lymph vessels; it is a chronic perilymphangitis.

O. T. SCHULTZ.

HYPERTONIC APOPLECTIC CEREBRAL HEMORRHAGE. K. WOLFF, Virchows Arch. f. path. Anat. **299**:573, 1937.

Following apoplectic cerebral hemorrhage in persons with a prolonged antecedent history of hypertension, a variety of alterations are observed in the arteries

in and about the hemorrhage. These include: angionecrosis, which accompanies and does not antedate the hemorrhage; a process which the author terms plasmatic destruction of the vessel wall, which precedes the hemorrhage and is associated with older changes in the brain tissue; arteriosclerotic changes that are likewise older than the terminal hemorrhage, and aneurysm subsequent to plasmatic destruction of the wall. Rupture of the diseased vessels leads to massive hemorrhage.

In persons without a prolonged antecedent history of hypertension the hemorrhage originates from many smaller, apparently normal vessels, which leads the author to conclude that functional circulatory disturbances of unknown nature initiate the hemorrhage. The latter is accompanied by angionecrosis of surrounding vessels. These then give way, and the compact massive hemorrhage results. No other alterations have been observed in the vessels. O. T. SCHULTZ.

CYTOPLASMIC INCLUSION BODIES OF THE HUMAN LIVER. A. TERBRÜGGEN, Virchows Arch. f. path. Anat. **299**:775, 1937.

Terbrüggen describes cytoplasmic inclusion bodies that he saw in 36 livers in 1930-1931. They were small and stained readily, and each was situated in a small vacuole. Their nature is not known. Their similarity to degeneration products, extruded nucleoli, virus inclusion bodies and protozoa is discussed. The inclusions are apparently identical with those described by Pappenheimer and Hawthorne (*Am. J. Path.* **12**:625, 1936). O. T. SCHULTZ.

VIRCHOW'S LECTURES ON PATHOLOGY AT WÜRZBURG. R. RÖSSLE, Virchows Arch. f. path. Anat. **300**:4, 1937.

The three-hundredth volume of *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, to give it the full title that Virchow selected to indicate the full scope of the new journal, has a brief foreword by Rössle, Virchow's successor as professor of pathology at Berlin and as editor of the journal. Begun in 1847 by Virchow and published uninterruptedly ever since, *Virchows Archiv* is the oldest journal devoted to medical science. Rössle points out that the only scientific journals older that are still being published are the French *Annals of Physics* (1789), the oldest scientific periodical in the world, the German *Annals of Physics* (1799) and Liebig's *Annals of Chemistry* (1834). The entire double number of volume 300, comprising 516 pages, is devoted to 27 articles based on work done in Rössle's institute in Berlin, the post which Virchow had made the outstanding medical position in the world. In addition to numerous halftone illustrations, the volume contains several reproductions of colored drawings reproduced directly on the printed page, a process developed more highly in German publications than in any others.

After his introductory foreword, Rössle proceeds with a contribution of historical interest. It relates to Virchow's period at Würzburg, from 1849 to 1856, when he, a young man of 28 years at the beginning of this period, began attracting students to Würzburg from all parts of Germany. The article is based on recently discovered manuscript lecture notes of students of Virchow at Würzburg and contains numerous excerps from these notes. Among the students whose notes have come to light and are quoted are Goll and Wilhelm His the elder. The lectures dealt chiefly with general pathologic anatomy, but there are notes of courses in special pathology, especially that of bone. Virchow had already begun to lecture to his students on cellular pathology, a subject which was later elaborated into his famous lectures on cellular pathology at Berlin. Complete publication of the student material that Rössle discusses is promised.

O. T. SCHULTZ.

ORIGIN AND FATE OF LYMPHATICS IN PARABIOTIC ANIMALS. R. RÖSSLE, Virchows Arch f. path. Anat. **300**:31, 1937.

In material derived from successful experiments in parabiosis on rats and mice, Rössle describes the formation of lymph vessels and adds an important contribution on the still unsettled matter of the origin of such vessels. At the line of junction of the two animals the formation of new lymphatics by budding from the original channels can be observed, but this is a process of minor importance. What more particularly concerns Rössle is the development of new lymphatics independently of preexisting vessels. Near the line of junction of the parabions, solid cords and masses of small cells with deeply stained nuclei make their appearance between the bundles of fibrous tissue. Whether these cells come from the nuclei of connective tissue or from wandering cells, Rössle is unable to decide. A lumen develops, and the cells become larger and cuboid, resulting in structures almost glandular in character. These new-formed lymphatics do not come from preexisting ones, and no connection with the lymphatic system of either animal of the united pair ever develops. In animals that live long enough coagulation, thrombosis and organization of the contents of the lymphatics occur, and the vessels become obliterated. This closed lymphatic system usually develops in the cutis and subcutaneous tissue of the larger and stronger parabion near the line of junction. Rössle terms this system a *Saugapparat*, or suction apparatus. It withdraws materials from the other parabion, materials which are foreign to the other partner, which stimulate the formation of the lymphatics and lead to the development of immunity, after which the new-formed closed lymphatics disappear. What Rössle finds more difficult to explain is the development of a closed lymphatic system in each parabion. He suggests that this may be due to alternating periods of dominance of one over the other.

O. T. SCHULTZ.

Microbiology and Parasitology

THE SUBMAXILLARY GLAND VIRUS OF THE GUINEA PIG. F. S. MARKHAM, Am. J. Path. **14**:311, 1938.

Spontaneous infection with virus from the submaxillary glands of guinea pigs is described. The incidence in various local stocks of guinea pigs was found to be from 7 to 74 per cent. Inclusions were found in the renal epithelial cells of 8 per cent of the adult animals examined. Histologic evidence is presented which suggests that the inclusion bodies associated with the virus of the salivary gland of the guinea pig are composed of elementary bodies similar to those known to occur in certain other virus diseases. The infectivity of the virus is greater for the fetus than for the postnatal guinea pig. Natural passive immunization in utero or per colostrum is inadequate to protect fetuses or suckling guinea pigs against experimental infection. In spontaneously infected adults the quality or duration of active immunity may depend on the presence of active lesions. An analogy between the distribution of the inclusion bodies sometimes found in stillborn and premature human infants and the distribution of such bodies in experimentally infected guinea pig fetuses is pointed out.

FROM AUTHOR'S SUMMARY.

EFFECT OF FORMALDEHYDE ON PNEUMOCOCCI. R. J. DUBOS, J. Exper. Med. **67**: 389, 1938.

When used in low concentration, formaldehyde increases the rate of autolytic disintegration of pneumococci, whereas in high concentration it completely inhibits autolysis and preserves both the morphologic and the staining characteristics of the cells. Pneumococci treated with formaldehyde in high concentration, then washed free from the antiseptic and resuspended in physiologic solution of sodium chloride rapidly undergo a change which renders them gram-negative and smaller.

The lysis is only partial, however, and is not accompanied by disintegration of the cell. It is caused by the autolytic enzyme of the cell, which remains inactive in the presence of an excess of formaldehyde but recovers its activity when the cells are resuspended in a neutral medium after removal of the antiseptic. If the autolytic enzyme is irreversibly inactivated by heating, or if it is maintained inactive in an acid or an alkaline reaction, the formaldehydized cells retain their staining characteristics and morphologic integrity. Formaldehydized pneumococci which have become gram-negative owing to the action of their autolytic enzyme fail to elicit antibodies for the type-specific carbohydrate when injected into rabbits. Formaldehydized pneumococci in which the autolytic enzyme has been destroyed or maintained inactive and which have retained their gram-positive character function as a very effective type-specific antigen in the rabbit. These observations emphasize once more the close relation between the gram-positive structure of pneumococci and the capsular polysaccharide antigen of the cell. They can be used as a basis for the preparation of suspensions of formaldehydized pneumococci which will be stable and effective as type-specific antigens.

FROM AUTHOR'S SUMMARY.

INHIBITORY SUBSTANCE FOR INFLUENZA ORGANISMS. E. KRUMWIEDE and A. G. KUTTNER, J. Exper. Med. **67**:429, 1938.

Five per cent sheep blood agar is a selective medium for beta hemolytic streptococci in throat cultures since sheep blood inhibits the growth of bacillus X (Haemophilus haemolyticus) and Bacillus parainfluenzae haemolyticus. The growth of Haemophilus influenzae is also inhibited by sheep blood. This inhibitory action resides in the erythrocytes and is thermolabile. The inhibitor is not affected by disruption of the erythrocytes in laking. A similar inhibitory action on the growth of hemolytic and nonhemolytic members of the influenza group is noted with blood from animals closely related to the sheep, such as the goat and the cow; human blood contains a similar but less powerful inhibitor. Members of the influenza group grow well on unheated rodent blood: rabbit, guinea pig and rat. These organisms also grow fairly well on unheated horse blood.

FROM AUTHORS' SUMMARY.

EFFECTS OF ACIDITY ON PNEUMOCOCCUS GROWTH. W. H. KELLEY, J. Exper. Med. **67**:667, 1938.

In the presence of animal fluids or their protein constituents, type I pneumococci survived and multiplied at acid hydrogen ion concentrations which in the plain broth were bactericidal for these organisms. Minimal numbers of these cells readily produced growth in serum broth when the broth was adjusted at a hydrogen ion concentration as great as p_H 5.5 with hydrochloric acid or to p_H 6.5 with acetic acid. Growth of the pneumococci could be demonstrated in serum broth adjusted to p_H 5 with hydrochloric acid or to p_H 5.5 with acetic acid, although at these hydrogen ion concentrations large amounts of inoculum were necessary. Similar results were obtained with broth to which certain animal proteins had been added and in serum broth which had been heated in the autoclave at 20 pounds' (9 Kg.) pressure for twenty minutes. Pneumococcus growth proceeded at a more rapid rate in serum-dextrose broth at p_H 6.5 than in dextrose broth at the optimal hydrogen ion concentration of p_H 7.8. At p_H 6 large numbers of pneumococci failed to produce the same amount of growth in serum-dextrose broth as at p_H 6.5 or in dextrose broth as at p_H 7.8. It is of interest that in cultures in serum-dextrose broth the stationary and decline phases of pneumococcus growth were prolonged, and cell death delayed, in comparison with cultures in dextrose broth alone.

FROM AUTHOR'S SUMMARY.

SATELLITE HEMOLYTIC ZONES IN BLOOD AGAR STAPHYLOCOCCUS CULTURES. G. B. RHODES, J. Infect. Dis. **62**:124, 1938.

From certain strains of growing hemolytic Staphylococcus aureus a substance is diffused which produces discrete hemolytic zones in blood agar plates. These occur only when unheated serum is in the medium and are unrelated to the complement content of such serum. The occurrence and the number and size of the zones vary with the serum and erythrocytes of different animals.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL INVESTIGATIONS IN HEMORRHAGIC ENCEPHALITIS. A. B. BAKER and C. W. BUGGS, J. Infect. Dis. **62**:293, 1938.

Fresh brain tissue obtained from a person who died of hemorrhagic encephalitis and injected intracerebrally into experimental animals proved very infectious to rabbits. Guinea pigs and white mice did not react to it. The active agent was passed serially through sixteen sets of animals before it was lost. All the animals presented the characteristic symptoms of an involvement of the central nervous system: hyperirritability, muscular twitchings, motor weakness, convulsions and moderate salivation. The active agent lost its potency when stored in 50 per cent glycerol at 0 C. for one month. Attempts to pass it through a filter proved unsuccessful. One instance is noted in which it proved infectious via subcutaneous inoculation. The brains of the inoculated rabbits revealed changes quite similar to those found in man in hemorrhagic encephalitis.

FROM AUTHORS' SUMMARY.

OBSERVATIONS ON LIVING VACCINIA AND ECTROMELIA VIRUSES BY HIGH POWER MICROSCOPY. F. HIMMELWEIT, Brit. J. Exper. Path. **19**:108, 1938.

A method is described by which the living chorioallantoic membrane may be examined microscopically by annular oblique incident illumination while it is still in situ in the egg, with its vascular and other anatomic connections still undisturbed. The method has been applied to the study of virus bodies within the living cells of the chorioallantoic membrane of the duck egg infected with ectromelia and vaccinia viruses. Observations on the nature of the ectromelia inclusion body are reported, and the formation of extracellular giant aggregates is described. The presence and distribution of vaccinal elementary bodies within the living cell have been observed from early stages of cell infection. The elementary bodies as they exist in the cell are contained within a matrix of low viscosity, and their partial release in "extrusion bodies" is described. It is concluded that the vaccinal elementary body represents the only virus structure which can be recognized in the living cell and that the Guarnieri bodies seen in stained preparations correspond simply to localized irregular collections of elementary bodies and do not necessarily represent all the virus in the cell.

FROM AUTHOR'S SUMMARY.

TUBERCULOSIS IN AMERICAN SCHOOLS AND COLLEGES. E. R. LONG, Tubercle **19**: 241, 1938.

Studies continued over a decade indicate that the proportion of elementary and high school children positive to the tuberculin test is steadily falling. In the less crowded communities the drop is more striking than in large, congested cities. The incidence of the positive result is generally significantly lower in rural communities than in urban areas, and differences within the same community are always found corresponding to differences in economic level. Important tuberculous disease is first encountered to an appreciable extent in high school students, in whom the combined incidence of latent and manifest disease varies from 1.5 to nearly 3 per cent, being higher in girls. Important disease demanding care occurs in from 0.5 to 1 per cent of these children. Active programs for the

early detection and control of tuberculosis in schools now operate throughout the country. The basic principles of these programs are mass tuberculin testing, roentgen examination of the positive reactors and provision of suitable care for those found to have lesions. In the colleges about 6 students per thousand have tuberculosis of the adult type. A marked variation occurs geographically, students from the great central portion of the country having a relatively low incidence of the disease. Tuberculosis is recognized as the most serious disease of the college period yet as insidious in onset, requiring routine mass measures for its detection. Most of the lesions now discovered are in the minimal stage. Students of medicine and nursing are known to be subject to a special hazard. The danger for medical students seems greatest during the third and fourth, or clinical, years, suggesting that tuberculosis acquired in the medical school is usually exogenous. Most nurses who are negative to tuberculin on beginning training become reactors during their course. The incidence of clinical tuberculosis is also proportionately high in nurses as compared with other professional or working groups at the same age, both during the period of training and in the first years after qualification.

Polymorphism of Rickettsias of Trachoma. A Cuénod and R. Nataf, Arch. Inst. Pasteur de Tunis **27**:1, 1938.

Rickettsias of trachoma vary from extremely minute forms to cells as large as from 1 to 3 microns. The larger forms are visible in unstained trachomatous material under the high-dry objective. They are highly refractile and appear like brilliant luminous pearls which may be spheroid, ellipsoid or rarely rhomboid. They occur in groups of from 12 to 15, encircling the nuclei of epithelial cells. Extracellularly they are chiefly in pairs resembling dumbbells or thick rods. These rickettsias stain reddish purple by the Giemsa method but do not stain with ordinary aniline dyes. These forms are found also in inoculated lice, guinea pigs and rabbits.

J. B. Gunnison.

Experimental Studies of Herpes Virus in White Mice. E. Gildemeister and I. Ahlfeld, Zentralbl. f. Bakt. (Abt. 1) **139**:325, 1937.

Gildemeister and Ahlfeld were unable to infect white mice orally or monkeys cutaneously with herpes virus (strain Basel III). Normal rabbit serum contained no protective antibodies against cutaneous infection of white mice with herpes virus. Active immunization of rabbits with this virus, however, led to development of neutralizing antibodies. The serum of many persons also contained protective antibodies against herpes virus, not only that from persons who had often suffered from herpes but also that from persons who had not had herpes. White mice could be immunized passively with rabbit antiherpes serum, but the immunity lasted only a few days. Such serum also had therapeutic value in mice suffering from herpes. The immunity of white mice following herpetic infection was of short duration.

Paul R. Cannon.

Bacillus Vaginalis of Döderlein as a Cause of Endocarditis. Fred Marschall, Zentralbl. f. Bakt. (Abt. 1) **141**:153, 1938.

A case of ulcerative endocarditis in a woman 21 years of age is described, in which a pure culture of acidophilic bacilli was obtained from the blood before death and another from the mitral valve after death. This micro-organism was identified by cultural and serologic tests as Bacillus vaginalis of Döderlein. It is considered by the author to have been the cause of the endocarditis because of the finding of the organism in a blood culture before death, the isolation of the organism in pure culture from several organs after death, the presence of agglutinins to the organism in the blood serum and the demonstration of the bacilli in the endocarditic lesions.

Paul R. Cannon.

Immunology

INTRACUTANEOUS REACTIONS AGAINST ANTISERUM IN TUBERCULOSIS. H. J. CORPER and C. B. VIDAL, Am. Rev. Tuberc. **37**:239, 1938.

Although it is conceded on the basis of prior experiments that there is a specific immunity to tuberculosis, demonstrable both in man and animals, it is found impossible to demonstrate the presence of such immunity by means of intracutaneous tests with specific antiserums. All attempts to produce an antiserum that would react specifically on intracutaneous injection into animals of the same species (in order to exclude foreign serum reactions) proved futile. Antiserums were prepared in guinea pigs, rabbits and dogs and were injected into animals infected with virulent and avirulent human tubercle bacilli. In human subjects, both normal and tuberculous, with negative and positive reactions to tuberculin (purified protein derivative), no specific reactions were obtained to antiserum. Serum from normal and from immunized goats produced intracutaneous reactions in normal and tuberculous human subjects. These reactions developed after from three days to two weeks and persisted for from several days to a week; they were of variable intensity.

H. J. CORPER.

IMMUNIZING SUBSTANCES IN PNEUMOCOCCI. L. D. FELTON and G. KAUFFMANN, Bull. Johns Hopkins Hosp. **62**:430, 1938.

The results of this study indicate that the amount of antigenic substance in the bacterial cells varies with growth. Young cultures demonstrated high antigenicity both of the bacterial cell and of the substance extracted from it; from old cultures, of low antigenicity, only a relatively small yield of active substance was obtained. The "essential immunizing antigen" of the pneumococcus has been defined as that substance isolated either from the cell or from the culture medium which contains as many immunizing doses for mice as the bacterial cells or fluid from which it is derived. Felton and Kauffmann have demonstrated the possibility that from pneumococci of types I and II fractions may be isolated which contain from four to eleven hundred times as many immunizing doses as do the original cells from which the fractions were made. This degree of activity is demonstrable, at least in white mice, by extracting directly from the cells the fraction soluble at p_H 3 in hydrochloric acid or in 5 per cent trichloracetic acid or by extracting the fraction after digestion of the cells with trypsin, pancreatin or papain. The weight of dried organisms of type I containing a million immunizing doses varied from 1 to 10,000 Gm., whereas the weight of the isolated fraction containing the same number of immunizing doses varied from 0.012 to 5 Gm. With organisms of type II, the corresponding weights were from 0.8 to 100 Gm. and from 0.01 to 0.5 Gm. At least 85 per cent of the bacterial cell was found to be inert. The chemical nature of this fraction was not entered into except for the dextrose number. The amount of this component did not correlate with the degree of antigenic activity. The authors describe an experiment with organisms of type I and another with organisms of type II in which after heating the organisms in hundredth-normal alkali at 100 C. for thirty minutes an alcohol-soluble fraction was obtained which was as active as any other fraction of the entire cell so far isolated.

FROM AUTHORS' SUMMARY.

BLOOD-GROUPING AND COMPATIBILITY. P. HOXWORTH and A. AMES, J. A. M. A. **108**:1234, 1937.

Hoxworth and Ames employed a modification of the technics of Vincent and Coca for the determination of blood grouping and compatibility. In the determination of grouping large drops of high-titered test serums, anti-B and anti-A were added to the left and right ends, respectively, of a glass slide. Blood obtained by puncture of a finger and defibrinated by whipping was added to each drop of

serum and mixed by means of a platinum loop. In matching bloods a drop of a 50 per cent suspension of the recipient's defibrinated blood in saline solution was mixed with one fifth of a drop of 50 per cent suspension of the donor's defibrinated blood. The mixture was agitated and observed after fifteen minutes. This method was employed for more than 400 transfusions, without a reaction. Agglutination was prompt and clearly defined, and the readings were in agreement with those determined by the older methods. This method obviated the need for venipuncture and for separation of cells and serum. The 1:5 mixture of the donor's and the recipient's blood was needed for the detection of universal donors with dangerous amounts of agglutinin for A or B recipients.

FREDERICK STENN.

AN IMPROVED AIR DRIVEN TYPE OF ULTRACENTRIFUGE FOR MOLECULAR SEDIMENTATION. J. H. BAUER and E. G. PICKELS, J. Exper. Med. **65**:565, 1937.

A description is given of the construction and operation of an improved type of air-driven ultracentrifuge, operating in a vacuum and suitable for the determination of sedimentation constants of protein molecules. The rotor of the centrifuge is made of a forged aluminum alloy; it is oval, measures 185 mm. at its greatest diameter and weighs 3,430 Gm. It carries a transparent cell located at a distance of 65 mm. from the axis of rotation and designed to accommodate a fluid column 15 mm. high. The rotor has been run repeatedly over long periods at a speed of 60,000 revolutions per minute, which corresponds to a centrifugal force of 260,000 times gravity in the center of the cell. At this speed no deformation of the rotor or leakage of the cell has been observed. The sharp definition of sedimentation photographs taken at high speed serves to indicate the absence of detectable vibrations in the centrifuge. When a vacuum of less than 1 micron of mercury is maintained in the centrifuge chamber, the rise in the temperature of the rotor amounts to only 1 or 2 C. after several hours' run at high speed. There has been no evidence of convection currents interfering with normal sedimentation of protein molecules in the centrifugal field. A driving air pressure of about 18 pounds per square inch (8 Kg. to 6 sq. cm.) is sufficient to maintain the centrifuge at a steady speed of 60,000 revolutions per minute. With a driving pressure of 80 pounds per square inch (36 Kg. to 6 sq. cm.) it can be accelerated to this speed in less than twenty minutes, and it may also be brought to rest in about the same length of time by the application of the braking system. The adaptation of Svedberg's optical systems to this centrifuge for photographically recording the movement of sedimentation boundaries is described.

FROM THE AUTHORS' SUMMARY.

REACTIONS OF ANTI-AZOPROTEINS SERUM. K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **67**:709, 1938.

Azoproteins have been prepared with azocomponents possessing two serologically active groups. On immunization with such antigens immune serums were obtained containing two separate, unrelated antibodies, each specific for one of the two groups and separable by absorption. In other cases one of the two structures was dominant in that antibodies were formed only toward this and not toward the other grouping. The specificity of the antibodies was in general found to be influenced to some extent by the presence of a second group in the antigen. The relevancy of these observations for antibodies directed against natural antigens has been noted.

FROM AUTHORS' SUMMARY.

STATISTICAL STUDIES OF VACCINE VIRUS. R. F. PARKER, J. Exper. Med. **67**:725, 1938.

A method has been described by which it is possible to estimate the number of particles of vaccine virus which are required to cause infection in the skin of a rabbit. The method consists essentially in injecting suitably diluted suspensions

of the virus intradermally into rabbits in series. The percentage of inoculations at each dilution giving rise to lesions was observed, and the data are subjected to appropriate statistical analysis. Several strains of vaccine virus, differing in their characteristics, have been studied, with the following results: A single particle of the virus prepared by the New York City Board of Health appears to give infection or inoculation. The same is true for the strain derived from it but cultured in a chick embryo–Tyrode solution medium for a prolonged period. This strain, as has been noted, has largely lost its ability to cause extensive necrosis in the rabbit's skin and causes generalized infection only exceptionally. From the results reported here it appears that the alteration in the character of the lesion must be traced to factors other than the reduced ability of the virus to establish a foothold in the animal organisms. In this respect the cultured virus appears to be the equal of the original passage virus. Similarly the Noguchi strain of virus is apparently capable of infecting when a single particle is properly introduced.

FROM AUTHOR'S SUMMARY.

IN VITRO ACTION OF IMMUNE RAT SERUM ON THE NEMATOIDE, NIPPOSTRONGYLUS MURIS. M. P. SARLES, J. Infect. Dis. **62**:337, 1938.

Preparasitic infective larvae were freed from bacteria adherent to the cuticula by treatment with a 0.1 per cent solution of mercuric chloride, were sealed with petrolatum between a sterile slide and a cover slip in small drops of serum or saline solution, were kept at room temperature and at 36 C. and were observed at frequent intervals with regard to the occurrence of precipitates and their development and survival. This test was made on 14 immune rat serums, 13 normal rat serums and saline solution. The immune serums were from rats repeatedly infected and had been proved to have an antibody content by their power to immunize normal rats passively. Also tested were parasites in the pulmonary and intestinal stages from rats, in 4 immune and 4 normal serums and in saline solution. In all 3 stages the parasites survived longer and were more active in serum than in saline solution; they survived about an equal time in normal and immune serums and in both were seen actively feeding by vigorous rhythmic contractions of the esophagus. Only infective larvae developed in vitro, passing through what corresponded to the parasitic phase of development in the skin, but their development was less marked in immune than in normal serum. Evidences of antibody action, seen with worms in immune serums but not with those in normal serums or saline solution, included: (1) invariable formation of precipitates of (*a*) cuticular type (with larvae and parasites in the pulmonary stage only), (*b*) excretory type, (*c*) oral type and (*d*) intestinal type and (2) sometimes decreased activity (of larvae and parasites in the pulmonary stage) and inhibition of development (of larvae). The correspondence of certain of these reactions to those seen in actively and passively immunized rats and their probable role in acquired immunity to Nippostrongylus muris are discussed.

FROM AUTHOR'S SUMMARY.

THE ISOLATION OF ANTIGENIC SUBSTANCES FROM STRAINS OF BACTERIUM TYPHOSUM. D. W. HENDERSON and W. T. J. MORGAN, Brit. J. Exper. Path. **19**:82, 1938.

When suitable strains of Bacterium typhosum are extracted with anhydrous diethylene glycol, substances are obtained which are apparently free from protein and which are antigenically active. In extracts from rough Vi strains Vi antigen can be detected as a chemical entity separate and distinct from O substance. From O agglutinable strains substances containing O antigen are readily isolated, but the authors have not succeeded in obtaining these preparations entirely free from Vi antigen. In extracts obtained from strains that are rich in flagella traces of H antigen have been detected. This finding was probably due to passage of flagellar

débris through the bacterial filters used in the preparation of the extracts. The process of extraction modifies the functional activity of the antigenic complex in rough Vi strains: Immunization with these extracts produces an immune body that is much less effective in protecting experimental animals than that produced by immunizing with living suspensions of rough Vi bacilli. The value of mucin as an adjuvant to the test dose of typhoid bacilli for experiments on typhoid infection in mice has been confirmed. FROM AUTHORS' SUMMARY.

THE FORMAMIDE METHOD FOR THE EXTRACTION OF POLYSACCHARIDES FROM HEMOLYTIC STREPTOCOCCI. A. T. FULLER, Brit. J. Exper. Path. **19**:130, 1938.

The formamide method for preparing group-specific extracts from hemolytic streptococci is described. It has the following advantages over existing methods: 1. It completely dissolves the bacteria, thereby giving potent extracts. 2. It destroys or removes protein substances that might give cross reactions.

A preliminary purification of the group-specific polysaccharides is described. It is suggested that the method may be applicable to all species of bacteria.

FROM AUTHOR'S SUMMARY.

THE PREPARATION OF SERUM PROTECTIVE AGAINST HEMOLYTIC STREPTOCOCCI. H. LOEWENTHAL, Brit. J. Exper. Path. **19**:143, 1938.

The antigen responsible for the production of the antibody effective against the invasiveness of hemolytic streptococci is intimately associated with the capsule which develops on these cocci in the early hours of growth in culture. This antigen is extremely labile, being readily destroyed by heat and by low concentrations of formaldehyde or of merthiolate. A temperature of 55 C. applied to a suspension of young encapsulated cocci for twelve minutes kills the organisms but leaves the capsular antigen intact. More prolonged application of this temperature rapidly destroys this antigen. With suspensions of young cultures which have been cautiously killed with heat, it has been possible for the first time to prepare a serum potently protective against hemolytic streptococci in the mucoid phase. This method of immunization also gives rise, more rapidly and regularly than do the old methods, to antibody protective against nonmucoid strains. The result of a single experiment suggests that it is possible to produce a potent polyvalent serum by immunizing with a number of strains simultaneously.

FROM AUTHOR'S SUMMARY.

RELATION OF THE COLLOIDAL STRUCTURE TO THE ACTION OF COMPLEMENT AND TO PAROXYSMAL HEMOGLOBINURIA. H. SACHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:328, 1937.

The ability of complement to lyse red blood cells on addition of silicic acid, snake venom, insulin or tannic acid in the absence of a hemolytic antibody suggests that the latter is not absolutely essential for the lytic action of complement. The supposition is made that the aforementioned substances substitute for the specific antibody. The observation that in a hypotonic solution complement alone may cause lysis forces one to conclude that colloidal phenomena and not substitution of nonspecific substances for the lytic antibody are at play. How can this be explained? Sachs suggests that the phenomenon is essentially the same as in the true antigen-antibody reaction. Here the globulin of the antibody forms a film on the surface of the antigen, and the action of the complement follows. The lysis without the lytic antibody can be explained as due to alteration of the globulin of the complement by the mentioned substances or by the hypotonicity of the medium. This concept makes it possible to explain paroxysmal hemoglobinuria without assumption of a hypothetic autoantibody. In this instance, chilling would be the cause of the alteration of the globulins in the patient's serum, and lysis would take place after the chilling was over. I. DAVIDSOHN.

IMMUNITY AGAINST PYOGENIC STAPHYLOCOCCI. Å. PETTERSSON, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:366, 1937.

The fatal dose when staphylococci were injected intravenously was from forty to one hundred times smaller than the dose by any other route. In the defense against staphylococci, leukocytes play the main part, as shown by the protective influence exerted by them when mixtures of leukocytes and staphylococci were injected into rabbits. The serum of animals inoculated with staphylococci did not inhibit leukocytic phagocytosis to any greater extent than normal serum did, but the leukocytes of immunized animals had greater phagocytic ability than the leukocytes of nonimmunized animals. Staphylococci induce negative chemotaxis in leukocytes, which keeps the latter away. That action is responsible for the edema and interferes with the formation of abscesses. Pettersson advocates that in the preparation of immune serums attention be paid to the formation of antibodies against the negative chemical influence of staphylococci.

I. DAVIDSOHN.

ELIMINATION OF GROUP SPECIFIC SUBSTANCES. P. DAHR and H. LINDAU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:470, 1937.

The substance A was detected in the saliva and urine of a chimpanzee and in the urine but not in the saliva of another chimpanzee; both apes belonged to group A. This report brings the number of grouped chimpanzees to 90, 78 of whom were classified as A and 12 as O. The group property B was shown to consist of three fractions—B_1, B_2 and B_3. The B_2 and B_3 fractions were found in various animal species, the B_1 only in man and in anthropoid apes. The distribution of the fractions B_2 and B_3 in animals is not always identical in the red blood cells and in the different secretions; for instance, in one rabbit the red blood cells had the fractions B_2 and B_3 and the saliva only B_3. In some monkeys the blood had only one fraction and the saliva two. The discrepancies in reports due to elimination of the property B in secretions on one occasion and the failure to find it on other occasions may be explained by the use of testing serums with different anti-B fractions.

I. DAVIDSOHN.

ISOAGGLUTININ ANTI-M. V. FRIEDENREICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **91**:485, 1937.

The blood serum of a 2½ month old baby whose blood was of group ON was found to contain the isoagglutinin anti-M. The mother's blood belonged to the same group but had no such isoagglutinin. As would be expected, the serum of the infant did not have anti-A and anti-B isoagglutinins. This serum containing the unusual anti-M agglutinin was equal in titer to the usual immune serums and reacted similarly in absorption experiments. The examination was repeated after one week with the same results. The father and 3 siblings of the mother were examined, with negative results. The observation was made in the course of litigation over the alleged paternity. Three men who were considered as possible fathers did not have the isoagglutinin anti-M in their blood. The child died at the age of 8 months, which prevented further investigation.

I. DAVIDSOHN.

DISTRIBUTION OF THE BLOOD GROUPS IN FINNS. U. P. KOKKO, Acta Soc. med. fenn. duodecim (Ser. A, no. 3, art. 14) **19**:1, 1937.

Blood specimens to the number of 1,518 were grouped by testing both cells and serum. Bloods from 1,334 Finnish-speaking persons in this series were distributed among the blood groups as follows: group O, 32.5 per cent; group A, 42.2 per cent; group B, 17.5 per cent, and group AB, 7.8 per cent. This agrees closely with previously published findings.

A. S. WIENER.

Tumors

EFFECT OF OIL OF WINTERGREEN ON SPONTANEOUS TUMORS OF THE MAMMARY GLANDS IN MICE. L. C. STRONG, Am. J. Cancer **32**:227, 1938.

Two fractions obtained by distillation of the true oil of wintergreen have different effects on spontaneous tumors of the mammary glands of mice. The high fraction has no effect on (1) the rate of growth of the tumors, (2) the time of survival of the animals or (3) the histologic structure of the tumors. The low fraction has a pronounced effect, causing (1) slowing of the rate of growth of the tumors, with complete regression in 4 of 34 animals, (2) increase in the time of survival after the onset of cancer and (3) gross and histologic alterations in the tumors. The action of the low fraction appears to be more pronounced than the action of the true oil of wintergreen. The difference in effect of the two fractions depends on the chemical difference between them and not on the methyl salicylate content which they have in common.

FROM AUTHOR'S CONCLUSIONS.

IMMUNIZATION AGAINST NEOPLASM: ITS EFFECT ON THE NITROGEN METABOLISM OF THE HOST. R. H. OSTER and W. T. SALTER, Am. J. Cancer **32**:422, 1938.

In 1925 Dodds found that certain rats which resisted inoculation with neoplasms showed a low concentration of urea in the blood after treatment with roentgen rays. Independently several investigators had shown that inoculation of tumors into mice may confer immunity to subsequent inoculation, an immunity which persists after removal of the tumors. This paper presents evidence that animals so immunized show the peculiarity in nitrogen metabolism which Dodds described in naturally immune animals. It suggests that susceptibility to malignant disease has a definite chemical background. The apparent concentration of urea in the blood of normal and tumor-bearing mice at the end of a period of fasting (twenty-four hours) is about 30 mg. per hundred cubic centimeters even after roentgen irradiation. Immune animals show the same value for urea until irradiated. Thereupon the apparent blood urea drops steadily for three days, to about 20 mg. per hundred cubic centimeters, and climbs back to normal in the course of the next week. The excretion of nitrogen in the urine shows urea to be consistently about 81 per cent and ammonia 9 per cent of the total nitrogen excreted on a mixed diet. This is true despite an excessive excretion of nitrogen during the day after roentgen treatment in both normal and immune animals. The drop in blood urea, therefore, is not due to a specific failure of the normal urea-producing mechanism. The response of apparent blood urea is independent of the actual presence of a tumor and indicates that the "immunity" is a property of the host primarily. Indeed, under certain special laboratory conditions, the chemical reaction may be used statistically to predict in average figures the approximate fate of inoculated neoplasms subsequently introduced. These observations suggest that the difference between a malignant and a benign tumor may reside in part in the chemical constitution of the host.

FROM AUTHORS' SUMMARY.

TUMOR GROWTH IN MICE ONE-FIFTH SATURATED WITH DEUTERIUM OXIDE (HEAVY WATER). H. G. BARBOUR and E. ALLEN, Am. J. Cancer **32**:440, 1938.

The growth of carcinoma in 7 mice and of lymphosarcoma in 2 mice was half as rapid, or less, in mice drinking 40 per cent heavy water (deuterium oxide) as in mice drinking ordinary water. Drinking 40 per cent deuterium oxide did not decrease the weight of young adult mice given injections of carcinoma or of lymphosarcoma. Mice receiving ordinary water grew faster, but much of the difference in weight could be accounted for by their larger tumors. Mice receiving deuterium oxide drank less than their controls receiving ordinary water by about a third or more. Survival of the tumor-bearing mice was shortened by deuterium oxide. Carcinomatous mice drinking 60 per cent heavy water failed to maintain

their body weight and died sooner than those receiving 40 per cent heavy water. It is not yet certain whether deuterium oxide exerts a specific action on the tumors in question by virtue of its osmotic properties or by virtue of its capacity to interfere with enzyme systems or whether the tumors are influenced by a growth-inhibiting property or merely by established catabolic effect of deuterium oxide.

FROM AUTHORS' SUMMARY.

AGENT AND SOIL IN EXPERIMENTAL CARCINOGENESIS. W. H. WÖGLOM, Am. J. Cancer **32**:447, 1938.

In the rat, relatively gross trauma does not lower the resistance of glandular epithelium to benzpyrine.

FROM AUTHOR'S CONCLUSION.

THE DEVELOPMENT OF SARCOMA IN MICE INJECTED WITH HORMONES OR HORMONE-LIKE SUBSTANCES. E. L. BURNS, V. SUNTZEFF and L. LOEB, Am. J. Cancer **32**:534, 1938.

Among 247 mice which had received injections of various endocrine preparations, including an extract of liver—most of them over relatively long periods of time—10 showed development of sarcoma. In 128 control mice, likewise observed over long periods, sarcoma did not appear. In 9 of the sarcoma-bearing mice ordinary spindle-cell sarcoma was formed; in 1 the rapidly growing tumor originated probably in striated muscle tissue. All the tumors except 1 developed at or near places where the tissues had been stimulated by the injections. Specificity of the stimulating factors is less pronounced in the case of sarcoma than in the case of epithelial proliferation induced by an estrogen, but the exact nature of the stimulating agent still needs to be determined. These tumors developed only after injections over a long period. While graded growth processes leading step by step toward cancerous growth can be recognized preceding the formation of epithelial tumors following injections of estrogenic substances, the growth of sarcomatous tumors seems to start suddenly. It is possible, however, that here, also, transitional growth processes precede the development of malignant growth.

FROM AUTHORS' SUMMARY.

A TRANSPLANTABLE UTERINE RAT SARCOMA OF 100 PER CENT TRANSMISSIBILITY. J. A. POLLIA, Am. J. Cancer **32**:545, 1938.

A new transplantable uterine sarcoma of 100 per cent transmissibility in the pure strain of animals from which it originated is described. In other standard strains the transmissibility is only about 20 per cent. The consistency with which the transplant "takes," the rapidity of growth and the inability to stop its development with any procedure other than irradiation make it a favorable medium for the study of the effect of alleged therapeutic agents on transplantable animal tumors.

FROM AUTHOR'S SUMMARY.

ROLE OF THE NEURAL CRESTS IN THE EMBRYONAL ADENOSARCOMAS OF THE KIDNEY. P. MASON, Am. J. Cancer **33**:1, 1938.

The three renal tumors studied in this work present the classic characteristics of embryonal adenosarcoma. Study of the tumors by ordinary and by neurologic methods shows that they are composed not only of the renal glandular elements, mesenchymatous elements and striated muscle admitted by the majority of authors but also of neuroepithelial and nerve elements which can be linked with the lateral lumbar neuroepithelium and the neural crests derived from it. Their "nephrogenous blastema" is composed of undifferentiated cellular cords formed by neuroepithelial vesicles. These cords have the structure of neural crests and give origin first to neuroblasts, especially of a sympathetic type, to lemmoblasts, to a mesectodermal mesenchyme and to striated muscle fibers. These same cords later dis-

appear in giving birth to rudimentary nephrons. The tumor blastema is, therefore, neurogenic, sclerogenic, myogenic and nephrogenic. Its neurogenic properties show the certain participation of the lumbolateral neuroepithelium and its crests in the construction of mixed tumors of the kidney. Its myogenic and nephrogenic properties raise a question: Are the lumbar crests the possible source of certain striated muscles and of the metanephrogenic blastema? The reply belongs to experimental embryology. If one reflects on the frequent presence of spinal and sympathetic ganglions in a number of teratomas composed of adult tissue, it is permissible to believe that these elements themselves come from neural crests but that these neural crests have existed as such only in the very earliest stages of the development of the tumors and have rapidly disappeared as they do in the normal embryo because they have become differentiated. It is only in malignant teratomas with immature tissues that one can hope to encounter them.

FROM AUTHOR'S SUMMARY.

"INFLAMMATORY CARCINOMA" OF THE BREAST. G. W. TAYLOR and A. MELTZER, Am. J. Cancer **33**:33, 1938.

A clinical study was made of 38 cases of inflammatory carcinoma of the breast encountered over a nine and one-half year period. This grave disease is not rare. The literature contains records of over a hundred cases. In the present series the incidence was 4 per cent of the total number of cancers of the breast. Although the disease is rare after the age of 70, its distribution with respect to age is the same as for cancer of the breast in general. The inflammatory signs may arise simultaneously with the cancer (primary type), or they may occur after a scirrhous cancer has been present for some time (secondary type). In the primary group a common early symptom is pain. The signs of inflammation may lead to a mistaken diagnosis and injudicious early therapy. The primary cancer of the breast comes to medical attention early, yet on admission the signs of inflammation are usually full blown and the disease widespread. The cancer may have an acute erysipeloid distribution, or it may show a tendency to nodular localization. It rarely ulcerates. It spreads rapidly in the superficial lymphatic structures of the thoracic wall. Multiple visceral metastases occur early, but the rapid course of the disease often does not permit them to attain clinical recognition. Metastases in bones were recognized roentgenologically in only 4 cases of the primary type. In the uncomplicated cases leukocytosis, fever and other signs of toxicity are rare. The patients maintain remarkably good health through the greater part of the course, and cachexia is unusual. Death is most often due to intrathoracic complications. The average duration of life in cases of the primary type was 21.3 months; in cases of the secondary type it was 10.8 months after the appearance of inflammatory signs. The inflammatory signs—edema, redness and heat—are due to extensive blockage of lymphatic channels by the cancer and to congestion of the subpapillary plexus. There is no uniform pathologic type. The large fatty breast seems predisposed, as does the hyperplastic breast of the woman whose pregnancy is far advanced or of the lactating mother. No other predisposing factors could be established. The results of therapy are poor. Surgical removal is followed by prompt evidence of supraclavicular disease, recurrence in the skin or invasion of the opposite breast. Roentgen therapy seems to give the best palliative results. Artificial menopause does not alter the course of the disease.

FROM AUTHORS' SUMMARY.

SPONTANEOUS BONE TUMORS OF MICE. F. C. PYBUS and E. W. WHITE, Am. J. Cancer **33**:98, 1938.

From 2 mice of Simpson strain 3 has been derived a selected inbred branch in which the incidence of bone tumors (sarcoma) is very high. A brief account is given of the main types and sites of these tumors and of their distribution with respect to age.

FROM AUTHORS' SUMMARY.

A Transmissible Leukemia in the "A" Strain of Mice. J. H. Lawrence and W. U. Gardner, Am. J. Cancer **33**:112, 1938.

Transmissible lymphatic leukemia has occurred in the Strong A strain of mice. It arose in a mouse receiving a prolonged course of injections of an estrogenic substance and has been transferred by subcutaneous and by intravenous injections of suspensions of minced spleen or lymph nodes, invariably giving 100 per cent "takes" and regularly causing death of the animals. The disease could not be produced by intravenous inoculation of cell-free filtrates. It could not be transmitted to mice of another strain.

From Authors' Summary.

Nesidioblastoma, the Islet Tumor of the Pancreas. G. F. Laidlaw, Am. J. Path. **14**:125, 1938.

Microscopically, the chief feature of nesidioblastoma in most of the instances is the exact duplication of the pattern of normal islets. The growths also resemble hypertrophied islets in their tendency to exaggerate some features of the normal islet pattern. Just as the tumors duplicate the structure of normal and hypertrophied islets, so they are subject to the same pathologic vicissitudes, such as fibrosis, hyaline degeneration and calcification. The origin of the tumor cells is indicated by the abundance of figures showing the epithelial lining of the duct continuous with a group of tumor cells. The origin of the name "nesidioblastoma" is explained.

From Author's Summary.

Transmission of Chloroleukemia of Mice. J. W. Hall and F. J. Knocke, Am. J. Path. **14**:217, 1938.

A strain of chloroleukemia of mice is described that was readily transmitted to related mice by intravenous injections of a suspension of the leukemic cells. Subcutaneous inoculation of the leukemic leukocytes produced localized tumors at the sites of inoculation in approximately 23 per cent of the inoculated mice. These tumors grew slowly. Intravenous injection of a suspension of the leukemic cells produced rapidly progressing generalized leukemia, fatal after approximately twenty days in 95.1 per cent of the mice receiving the cells. This observation indicates that large numbers of leukemic cells were destroyed in the subcutaneous tissues of mice that were susceptible to intravenous administration of similar cells. Suspensions of tumor cells injected intravenously were much less effective in transmitting the disease than spleen and lymph node. Tumor tissue and splenic tissue subcutaneously injected were about equally effective in producing subcutaneous tumor nodules. Exposure of mice to 400 roentgens preceding the injection resulted in a greater percentage of successful subcutaneous inoculations. Unrelated mice of two different stocks were resistant to transmission of the disease. Exposure of these mice to 400 roentgens has not rendered them susceptible to the disease. Mice have been negative following intravenous reinjection of leukemic splenic material. The almost complete absence of eosinophils in the leukemic infiltrations indicates that these cells are not responsible for the green color. The most intense green color is shown by the lymph nodes, while the subcutaneous tumors, which are composed almost exclusively of malignant leukemic cells, are gray with only a faint greenish hue.

From Authors' Summary.

Carcinogenic Effect of Papilloma Virus on Tarred Skin of Rabbits. P. Rous and J. G. Kidd, J. Exper. Med. **67**:399, 1938.

When the virus of the Shope papilloma is distributed by way of the blood stream to the tarred epidermis of domestic rabbits, it elicits carcinoma forthwith, as well as papilloma in great variety. The phenomenon will be analyzed, with the aid of additional instances in succeeding papers.

From Authors' Summary.

COURSE OF VIRUS-INDUCED RABBIT PAPILLOMAS AS DETERMINED BY VIRUS, CELLS AND HOST. J. G. KIDD, J. Exper. Med. **67**:551, 1938.

An experimental analysis of the factors responsible for the observed differences in the course of virus-induced papillomas in rabbits has shown that some of the factors are referable to the virus, others to the cells and yet others to the host. The interplay of these factors affords enlightenment of the nature of the cell-virus relationship in virus-induced tumors. Retrogression of the papillomas appears to be consequent on generalized resistance of host origin, elicited by and directed against the proliferating virus-infected cells.

FROM AUTHOR'S SUMMARY.

UTERINE ADENOMA IN THE RABBIT. H. S. N. GREENE and J. A. SAXTON JR., J. Exper. Med. **67**:691, 1938.

Eighty-three cases of an adenomatous tumor of the uterine mucosa have been observed in a colony of rabbits during the past four years. The results of a clinical and pathologic study of the tumor, together with a description of transplantation experiments, are included in the present report. The clinical histories of the tumor-bearing animals are similar: A long period of reproductive disturbance precedes the discovery of the tumor, and the tumor shows slow, continuous growth, with metastasis, terminating in death, in all animals held under observation for longer than one year. Microscopically, the tumor shows an atypical alveolar structure and in its characteristics closely resembles adenocarcinoma of the uterine fundus in women. Pathologic changes similar to those observed in mice following treatment with estrogenic substances are observed in the thyroid, adrenal, pituitary and mammary glands. Intraocular transplantation of the tumor has been successful, and at the present time the growth has been carried through six generations by serial transfer.

FROM AUTHORS' SUMMARY.

ECTODERMAL LESIONS PRODUCED BY THE VIRUS OF ROUS SARCOMA. E. V. KEOGH, Brit. J. Exper. Path. **19**:1, 1938.

The virus of Rous sarcoma has been propagated for thirty generations on the chorioallantoic membranes of developing chicks. In the chorioallantois the virus gives rise to purely ectodermal focal lesions. When dilute suspensions of the virus are inoculated, mixed mesodermal and ectodermal lesions arise. The virus may be titrated by enumerating the discrete lesions. Following inoculation of emulsions of passage membranes bearing discrete ectodermal lesions into fowls, typical Rous sarcoma appears.

FROM AUTHOR'S SUMMARY.

TUMORS IN RATS AND MICE FOLLOWING INJECTION OF THOROTRAST. F. R. SELBIE, Brit. J. Exper. Path. **19**:100, 1938.

Tumors can be readily induced in rats and mice by subcutaneous injection of thorium dioxide. Of rats surviving fifty-two weeks after the injection of 0.6 cc. of thorium dioxide, tumors were present in 58 per cent, and of mice surviving fifty-two weeks after the injection of 0.2 cc. of thorium dioxode, tumors were present in 26 per cent. It is suggested that the carcinogenicity of thorium dioxide is due not only to its radioactivity but also to the susceptibility of the inflammatory tissue which it produces.

FROM AUTHOR'S SUMMARY.

EFFECT OF X-RADIATION ON THE BLOOD AND LYMPHOID TISSUE OF TUMOR-BEARING ANIMALS. J. R. CLARKSON, W. V. MAYNEORD and L. D. PARSONS, J. Path. & Bact. **46**:221, 1938.

The growth of a mouse sarcoma was more rapid and the tumor of larger size in generally irradiated than in nonirradiated rats. Grafts into successive generations showed a higher percentage of "takes" in irradiated than in non-

irradiated rats. Investigation into the cause of the mortality of irradiated animals showed that general irradiation produces diminution in the lymphoid tissue throughout the body and that in lethal or sublethal doses it causes marked anemia, with structural changes in the lymph glands, more particularly the mesenteric, fatty changes in the liver and kidneys and atrophy of the spleen. The structural changes in the lymph nodes appear to convert these from normal lymph into hemolymph glands, concerned with phagocytosis of red cells. The deposit of iron in the tissues of irradiated animals and in those bearing primary tumors is discussed with special reference to the destruction of blood found in the lymphoid tissue of irradiated and nonirradiated mice in which sarcomas developed after treatment with a chemical compound.

FROM AUTHORS' SUMMARY.

VARIETIES OF CARCINOMA OF THE UPPER LIP. J. DELARUE AND C. FAYEIN, Bull. Assoc. franç. p. l'étude du cancer **27**:8, 1938.

Since 1922 carcinoma of the upper lip has been seen in 32 patients at the Cancer Institute of the Faculty of Medicine, in Paris. In 18 of these the growth originated in the cutaneous part of the lip; in 3 it grew from the adjacent skin into the lip. In all the patients except 1 with a spinocellular carcinoma, which metastasized to the lymph nodes, the carcinomatous growths responded very well to roentgen therapy. In 5 instances the carcinoma developed from the mucous membrane, and in 6 it straddled the mucocutaneous junction. This type showed more marked histologic irregularities than the cutaneous type; in most of these instances the carcinoma metastasized to submaxillary lymph nodes; in a certain instance it metastasized also to the preauricular lymph node on the side of the tumor. In the cases of carcinoma of the upper lip originating from the skin the growth behaved like cancer of the face in general. It was not related to sex. Metastases were rare; local roentgen therapy was highly successful. The prognosis in such cases is good. Carcinoma of the mucosa behaved like cancer of the buccal mucosa. It was more common in man (11 cases in a series of 12). It grew and metastasized rapidly. The rarity of carcinoma of the upper lip is evidenced by the fact that in the period during which 200 cases were observed in which carcinoma originated from the mucous membrane of the lower lip only 11 cases were encountered in which it originated from the upper lip.

I. DAVIDSOHN.

LYMPHATIC RETICULOENDOTHELIOMA OF THE UTERUS. J. L. NICOD, Bull. Assoc. franç. p. l'étude du cancer **27**:14, 1938.

In an enlarged uterus that was removed following a diagnosis of fibroma, the fundus appeared diffusely thickened, but there was no circumscribed tumor. Histologic sections revealed a network of lymph vessels lined with flat endothelial cells and filled partly or even completely with proliferating large cells, containing dark nuclei and abundant, occasionally vacuolated cytoplasm. In a few places, syncytium-like masses were present. Mitotic figures were absent. Nicod found only one similar, but not identical, case reported in the literature.

I. DAVIDSOHN.

SEMINOMA OF THE TESTIS. J. H. DEITERMANN, Frankfurt. Ztschr. f. Path. **50**: 231, 1937.

Deitermann reviewed 27 cases of seminoma of the testis from the standpoint of histologic characteristics and clinical malignancy. He could find no support for the theory that these growths are teratomatous in origin. Their malignancy is unpredictable from the histologic appearance except that in general they are less malignant than carcinoma of the testis. Thirty-seven per cent of the patients were living and well at the end of five years.

L. OHRINGER.

A Solitary Amyloid Tumor of the Parietal Bone. U. Buergi, Frankfurt. Ztschr. f. Path. **50**:410, 1937.

A 59 year old man had a fist-sized tumor of the parietal bone, which followed a traumatic injury of the skull and was diagnosed clinically as sarcoma. Roentgen treatment was ineffective. The tumor was removed, and the patient died. The tumor was chiefly composed of non-nucleated irregular structures, surrounded by granulation tissue containing many capillaries. These structures gave all the staining reactions for amyloid. The zones at the border of the tumor revealed that the primary deposition of amyloid occurred in the bone marrow and in the walls of the arteries of the bone marrow. No amyloid was present in any other organ. The author suggests that the deposition of amyloid was provoked by the traumatic lesion of the parietal bone. Similar tumors reported in the literature involved chiefly the vertebrae, ribs, sternum and skull. Tumors of this description are resistant to roentgen treatment and can be diagnosed only on biopsy.

Annemarie Strauss.

Neoplastic Character of Leukemia. K. Apitz, Virchows Arch. f. path. Anat. **299**:1, 1937.

In the course of one year there came to necropsy in Rössle's institute 15 persons dead of myelosis or lymphadenosis; in 7 (4 with myelosis and 3 with lymphadenosis) these conditions were not associated with tumor formation. In the remaining 8, diffuse myeloid or lymphoid hyperplasia or leukemic infiltration of the internal organs was associated with local tumor formation. The observations on these, together with the results of biopsy of tissue removed from 2 other persons, form the basis of Apitz' thesis that leukemia whether considered as myelosis or as lymphadenosis is a malignant neoplastic condition rather than a non-neoplastic hyperplasia of the myeloid or lymphoid tissues. As criteria of malignancy he stresses metastasis and aggressive growth. The 10 cases described and discussed in great detail fall into two main groups. The first comprised 3 cases of myelosis and 3 of lymphadenosis in which there developed tumor-like masses of malignant character or local aggressive proliferation. The second group consists of 4 cases of lymphosarcoma with terminal lymphadenosis of the leukemic type, held by many to be a systemic disease. The first 3 cases of the first group are held to be cases of myelosis with terminal development of myelosarcoma; the second 3 of the first group, cases of lymphadenosis terminating in lymphosarcoma. The cases of the second main group are held to be instances of lymphosarcoma with metastatic lymphadenosis. Apitz maintains that his investigations establish the neoplastic character of the myeloses and lymphadenoses, usually included under leukemia. The appearance of the proliferated cells in the peripheral blood is of clinical significance but not of essential differential importance. He proposes the following purely descriptive classification:

I. Lymphoid hemoblastoses

1. Lymphosarcoma of localized type capable of forming hematogenous or lymphogenous metastases (Ghon-Roman type)
2. Lymphosarcomatosis with regional involvement and without a recognizable primary focus (Kundrath-Paltauf type)
3. Lymphosarcoma with metastatic lymphadenosis
4. Lymphadenosis with terminal development of lymphosarcoma
5. Simple lymphadenosis, in which the aggressive character of the growth is evident only microscopically
6. Combinations of lymphosarcoma and lymphadenosis in which it is impossible to determine which was the primary condition.

II. Myeloid hemoblastoses

1. Myelosis with later development of myelosarcoma
2. Myelosis with localized aggressive growth or metastatic nodules (chloroleukemia)
3. Simple myelosis, in which aggressive growth is detectable only microscopically.

O. T. SCHULTZ.

THE IMMUNOLOGIC RELATIONSHIP BETWEEN THE SHOPE FIBROMA VIRUS, THE VIRUS OF MYXOMA AND NEUROLAPINE VIRUS. KO-DA GUO, Zentralbl. f. Bakt. (Abt. 1) **139**:308, 1937.

Experiments showed a definite relation between the neurovirus obtained from rabbits (neurolapine) and fibroma virus but none between the former and the virus of myxoma. Rabbits which had withstood a cutaneous invasion by the neurovirus obtained from rabbits exhibited local immunity to infection in the same area with the fibromatosis strain of the Shope fibroma virus, but when the latter virus was injected into other areas of skin infection developed. Rabbits which had withstood a cutaneous infection with neurolapine virus were also immune to infection with the inflammatory strain of the Shope fibroma virus but not to a later infection with the virus of infectious myxomatosis. A previous infection with Shope fibroma virus engendered no resistance to infection with myxoma virus.

PAUL R. CANNON.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Nov. 14, 1938

EDWIN F. HIRSCH, *Secretary*

PREPARATION OF M AND N TESTING FLUIDS. I. DAVIDSOHN.

In this paper I present my experience in the preparation of anti-M and anti-N immune serums and testing fluids. The details of the technic found useful are given. Boiled blood of type M (OM, A_1M and BM) was found well suited for the preparation of anti-N testing fluids. It can replace entirely raw blood of type M. On the other hand, for the preparation of anti-M testing fluid raw blood of type N must be used, because the latter does not resist boiling.

Anti-M and anti-N immune serums and testing fluids were kept frozen without preservatives for over six months without deterioration. Their value in the determination of the blood types M, N and MN for exclusion of paternity was discussed.

The report will be published in full in the *American Journal of Clinical Pathology*.

CONGENITAL ABSENCE OF THE PENIS. GEORGE J. RUKSTINAT and ROBERT HASTERLIK.

A male baby, 41 cm. long and weighing 2,020 Gm., born prematurely with a footling-breech presentation, had the following abnormalities: absence of the anus, penis and prostate gland and of the median raphe of the scrotum; a persistent atretic communication between the base of the bladder and the blind end of the distal part of the colon, which was hugely dilated; extensive fibrous and cystic changes of the kidneys; stenosis of both ureters; clubfoot on the left; displacement of the ossification centers of the transverse processes of the fifth lumbar and upper three sacral vertebrae. The right kidney measured only 8 by 10 by 10 mm.; the left, 15 by 12 by 10 mm. Both were nodular with cysts, which comprised about a third of the parenchyma. The remainder of each kidney consisted of heavy fibrous connective tissue strands which ensnared groups of glomeruli and collecting tubules. Most of the glomeruli were markedly altered and had thickened Bowman capsules and shrunken capillary tufts. The dilated renal tubules contained numerous lymphocytes mixed with débris and green globular material resembling the bile in meconium. The ureters were narrowed, the right throughout most of its length and the left near the ureteropelvic junction for 3 mm. The wall of the urinary bladder was up to 4 mm. thick. The lining was trabeculated and had four diverticulums, about 3 mm. deep, near the fundus. On the right side, the bladder bulged sharply at a point 2 mm. superior and anterior to the ureteral opening and communicated by a passage, 0.5 mm. in diameter, with the distal part of the colon. The opening was occluded by the wrinkled mucosa of the bladder but could be forced with gentle pressure. The adherent colon was 5.5 cm. in circumference and had a wall 0.5 mm. thick. The rugae of the lining occurred at wide intervals; the mucosa was stained with bile. The ascending part of the colon, by contrast, was only 14 mm. in circumference and 1 mm. thick. The distal end of the colon was smoothly rounded and had no distal outlet except at the narrow passage into the bladder. There was no vestige of a penis either externally or internally. The left testicle was in the scrotum, and the right was at the middle of the right psoas muscle.

DISCUSSION

P. GRUENWALD: Cases of atresia of the anus and absence of the penis are of great importance since Feller and Sternberg published their paper on sirenoid malformations, in 1931. Such malformations are defined as developing from median and symmetric defects of the anlage of the caudal end of the body. The group described was named after the best known subgroup, the sirens, which are characterized by a fusion of the legs. Feller and Sternberg have already stated, in their first report, that those defects can give origin not only to malformations characterized by fusion of the legs but also to many other anomalies of the pelvic region, e. g., atresia of the anus or absence of the penis. I think that the knowledge of the different types of sirenoid malformations will be helpful in the clarification of many of the various malformations of the pelvic region. Therefore the case presented has great importance.

O. SAPHIR: Were there any changes in the testes and what was the condition of the seminal vesicles and prostate?

S. LEVINSON: Is there a report of congenital absence of the penis without other malformations?

G. RUKSTINAT: Hemorrhages were the only changes noted in the testes. The prostate and seminal vesicles were absent. One report described this as the only anomaly in a boy. The ureters opened into the bowel. There are only twelve other reports of this malformation.

INTRACYSTIC PAPILLOMA OF THE BREAST. OTTO SAPHIR.

While minute intracystic papillomas of the breast often are present in instances of so-called chronic cystic mastitis, they also occur as independent tumors in breasts which have no other changes. Three varieties of intracystic papilloma may be differentiated: the fibrous type, the glandular type and a papilloma which consists of an insignificant connective tissue stalk covered by transitional epithelial cells resembling the mucosal lining cells of the urinary bladder (transitional cell type). A combination of these three types is seen frequently. The fibrous type is formed by a new growth of connective tissue which extends into the duct or cyst and is covered by duct lining cells. Such a tumor may be uniradicular or multiradicular. In multiradicular papilloma the epithelial cells of two adjacent rami may fuse, thus forming glandular structures. Possibly the glandular type is formed also by extension of neighboring periductile acini into a duct or cyst, the acini still being surrounded by the epithelial lining cells of the duct. Intracystic papilloma of the breast as a rule is multiple. The multiplicity may be explained on the basis of multiplicity of origin or by implantations of tumor cells in neighboring ducts. Such implants do not indicate necessarily that the tumor is malignant; the latter may be compared to certain benign ovarian tumors which occasionally produce implantations on the peritoneal surface. The multiplicity of intracystic papilloma of the breast and the consideration of the possible mode of origin of the secondary tumors should influence the choice of surgical procedure.

DISCUSSION

I. DAVIDSOHN: Intracystic papilloma and chronic cystic mastitis have been associated with disturbances of the glands of internal secretion, i. e., with the presence or absence of hormones. Two tissues, the stroma and the epithelium, are concerned, each specifically. Is hyperplasia of the epithelium present in young and absent in older patients, and were tall acidophilic acinar cells noted in the breast tissues in pregnant and not in nonpregnant women? Preparations of gonadotropic substances now being used cause changes of the mammary gland which are not spontaneous but are due to therapy.

O. SAPHIR: Papillomas are an integral part of chronic cystic mastitis. The ages of the patients whom I studied ranged between 20 and 50 years. I did not observe acidophilic cells.

MALACOPLAKIA OF THE URINARY BLADDER. ALEX B. RAGINS and DORRIN F. RUDNICK.

Malacoplakia of the urinary bladder is reported in a woman, aged 53 years. The lesions were associated with chronic ascending pyelonephritis, nephrolithiasis and chronic ureteritis. The plaques contained large polygonal cells with Michaelis-Gutmann bodies and also large accumulations of lymphocytes and plasma cells. They were entirely distinct from the transitional epithelium covering them, as shown by various differential stains.

Bacteriologic studies revealed the chief organism to be Bacillus mucosus capsulatus of Friedländer, which was isolated from the urinary bladder and the left ureter. On removal of the left kidney and ureter, all of the symptoms and the malacoplakia disappeared.

KATHARINE M. HOWELL, *President*

Regular Monthly Meeting, Dec. 12, 1938

EDWIN F. HIRSCH, *Secretary*

INGUINAL GRANULOMA WITH VISCERAL AND OSSEOUS LESIONS. ELEANOR M. HUMPHREYS.

The clinical and autopsy observations in a case of inguinal granuloma seem to contradict the widely held view that this disease is invariably superficial and local, accompanied by only mild general reactions. Search of the literature and a review of the few autopsy reports reveal only scanty evidence for the occurrence of generalized infection due to dissemination of the agent responsible for inguinal granuloma. In the case reported now, however, the highly characteristic Donovan organisms were demonstrated in the typical superficial lesions of the external genitalia and vicinity and in numerous lesions in the abdominal viscera, abdominal lymph nodes and bones of the thorax. The sites of these granulomatous and focally suppurative lesions were such as to indicate that they were caused by direct and deep extensions from the superficial lesions and by spread of the infectious agent through lymph and blood channels. There is no evidence that secondary bacterial infection played more than a minor role in the disease. Whether or not the pathologic observations in this case represent a rarity or exemplify merely the outcome in cases of advanced untreated inguinal granuloma will be determined only by more and careful postmortem studies.

DISCUSSION

E. A. PRIBRAM: What was the condition of the blood?

J. D. KIRSHBAUM: Was there amyloidosis?

ELEANOR M. HUMPHREYS: There was no amyloidosis. The erythrocytes of the blood were 4,120,000 and the leukocytes 14,000 per cubic millimeter just before the use of sulfanilamide. A differential examination was not made. After the use of sulfanilamide the erythrocyte count dropped to 1,200,000 and 2,000,000, and the leukocyte count, to 5,700.

HYPERNEPHROMA OF THE OVARY. F. W. VAN KIRK JR. and E. A. EDWARDS.

In this paper a discussion of opinions concerning the origin and classification of tumors of the ovary designated hypernephroma introduces the description of a left ovarian ovoid mass, 23.5 by 20 by 12 cm., which weighed 6 pounds and 2 ounces (2,778 Gm.). The patient, aged 42 years, had as her chief complaints

frequency of menstruation, constipation and a growing tumor mass in the abdomen. There were no changes of the secondary sex characteristics. Nineteen months after the operation there was no recurrence. All clinical observations indicated that the tumor was primary in the left ovary. On surfaces made by cutting the tumor there were a few cystlike pockets, ranging from 3 to 6 cm. in diameter, in a solid tissue composed of gray and yellow portions, the latter necrotic. The tumor tissues had large pale granular cells with sharp cell borders, arranged in alveoli and was like renal hypernephroma. The cytoplasm contained abundant glycogen. The glycogen content of the moist tissues on fixation in solution of formaldehyde U.S.P. was by chemical analysis 1.78 per cent.

DISCUSSION

WALTER SCHILLER: Among the hypernephroid tumors is the true hypernephroma, which originates either from the cortex of the adrenal gland in the normal location or from an island of adrenal cortex misplaced by developmental error into the cortex of the kidney. Such a neoplasm is real adrenal hypernephroma. There is another type of tumor, originating from the tubular region of the kidney and consisting of large polyhedral cells with a protoplasm full of fat droplets, which sometimes almost duplicates the true hypernephroma in appearance but otherwise has a marked tendency to form adenomatous or cystic structures and papillary projections. This type, since it originates from the renal cortex and secondarily duplicates only the structure of the hypernephroma, as Stoerk described, should not be called hypernephroma but simply hypernephroid. In the ovary both types of tumors can be found—the hypernephroma and the hypernephroid tumor. The case of Ruston, because of the marked papillary structure of the tumor, should be classified as an instance of hypernephroid.

The differentiation between the hypernephroma and the luteinized granulosa cell tumor can be made not only by comparison of the tumor cells but by analysis of the surrounding connective tissue. In the granulosa cell tumor the surrounding connective tissue has close functional and morphologic relations to the tumor tissue, duplicating the relation between the physiologic theca and the physiologic granulosa. In the adrenal cortex the framing connective tissue forms a neutral capsule only, which is not reactive to the activities of the included cortical tissue. The same holds for the hypernephroma. The tumor demonstrated by Dr. Van Kirk shows the absence of any but mechanical relations between the connective tissue capsule and the tumor tissue.

O. SAPHIR: The origin of some of the cells in the ovary is not exactly known and hence the origin of a tumor which arises in this tissue is not certain. I believe the differentiation between the true hypernephroma and the luteoma should be on the basis of biologic assay.

SYSTEMIC MYCOSIS WITH MYCOTIC ENDOCARDITIS. NATHAN B. FRIEDMAN and LILIAN DONALDSON.

A 57 year old Illinois grocer suffered from malaise and afternoon fever for a year before his death. Physical examination led to a diagnosis of subacute bacterial endocarditis, but the blood cultures were negative. The autopsy showed a luxuriant vegetation on the aortic valve, which microscopically proved to be filled with nests of yeastlike cells, and scattered through many organs were miliary tuberculoid granulomas.

DISCUSSION

E. A. PRIBRAM: One does not know how to classify this organism, because it was not identified culturally. Is there any possibility that the disease was tularemia?

O. SAPHIR: Perhaps the yeastlike organism is a secondary invader, engrafted on some other infection.

PRIMARY ADENOCARCINOMA OF THE JEJUNUM WITH PERFORATION. MAURICE B. JACOBS and E. A. CHRISTOFFERSON.

Primary carcinoma of the jejunum is rare. An acute perforation is still more unusual. Only one case has been reported heretofore in the last eight years. The present instance concerns a white man, aged 48 who was admitted to the Cook County Hospital on May 1, 1935, complaining of sudden severe abdominal pains. There had been no previous complaints referable to the gastrointestinal tract. His temperature was 100.6 F. rectally; the pulse rate, 102; the respirations, 36 per minute, and the blood pressure, 132 systolic and 80 diastolic. The abdomen was hard, slightly distended and diffusely tender on palpation. Peristaltic sounds were absent except for an occasional metallic click. Fluoroscopic examination revealed free air beneath the right dome of the diaphragm. A preoperative diagnosis of perforated peptic ulcer was made. Twenty-six hours after the onset of pain, a perforation of the jejunum 18 inches (45.7 cm.) distal to the ligament of Treitz was found. The regional lymph nodes were firm and enlarged. A diagnosis of carcinoma was made, and wide resection of the involved tissues together with the mesentery was done. Histologic examination revealed the tumor mass in the jejunum to be adenocarcinoma. Four days after the operation the patient died, and the postmortem examination demonstrated generalized fibrinous peritonitis and metastases in the mesenteric and periaortic lymph nodes. Both lower lobes of the lungs showed bronchopneumonia.

The literature was reviewed, and only one other case, observed by Dencks, was found recorded. In 10,309 consecutive necropsies at the Cook County Hospital between 1929 and 1938 only 7 cases of carcinoma of the small intestine were encountered, of which 3 were in the duodenum and 4 in the jejunum. Combined statistics from hospitals of Vienna, Baltimore and Chicago, covering 90,937 necropsies, included only 58 cases of primary carcinoma of the small intestine and in only 8 of these was the growth in the jejunum.

DISCUSSION

ALEX B. RAGINS: Up to 1938, 3 cases of malignant tumor of the small bowel in addition to the 7 cases described by Drs. Jacobs and Christofferson were observed at the Cook County Hospital. Two of these were instances of reticulum cell lymphosarcoma. In the first the tumor was located in the second portion of the duodenum, involving the ampulla of Vater, and in the second the tumor involved portions of the entire small bowel, starting from the jejunum, extending through the ileum and ending abruptly at the ileocecal junction. The third tumor was a pedunculated leiomyosarcoma of the ileum. Since observing these, I have encountered 3 more instances of sarcoma of the small bowel in which the growth had been resected surgically. In one the tumor was in the jejunum and in the others it was in the ileum. In the former the growth was lymphosarcoma. In the latter 2 it was reticulum cell lymphosarcoma. My associates and I have found carcinoma occurring more frequently in the small intestine, where most investigators have shown a marked prevalence of lymphosarcoma over carcinoma.

Book Reviews

The Special Pathological Anatomy and Pathogenesis of the Circulatory, Renal and Digestive Systems Including the Liver, Pancreas and Peritoneum. Horst Oertel, Strathcona Professor of Pathology, Director of the Pathological Institute, McGill University, and Pathologist to the Royal Victoria Hospital, Montreal, Canada. Pp. 630. Price $8.50. Montreal, Canada: Renouf Publishing Company.

This book is the outgrowth of a series of notes for the author's lectures, which have been expanded, elaborated and clarified in Professor Oertel's usual scholarly style. The preface and introduction are philosophic essays, based on wide reading and experience in educational problems, well worthy of perpetuation in this or any other form. The text is written with historical perspective. Emphasis is laid on etiology and pathogenesis, and there is furnished a thorough survey of pathologic morphology and function. For Oertel, pathology is a science of and for itself, but even so he is familiar with its practical bearing, and the facts and discussions are so presented that the book has genuine value for both the clinician and the pathologist. It should also be useful to those among the medical students whose interest is in the subject rather than merely in the final examination. The book reads smoothly, and the matter is fluently put, but it must be said that the writing is by the large, as Oertel says, "discursive." This formula gives him ample opportunity for full critical and informative discussion. Clarity, thoroughness and balance in the treatment of the subject matter prevail throughout.

Special or systemic pathology is of the deepest interest to those who would use this body of knowledge in the practice of medicine. Special pathology, however, in order to be sound, must be built on a solid foundation of general pathology, and most important in this broad field is knowledge of inflammation, of its nature and processes. Oertel is an adherent of Ricker's hypothesis of the nervous control of inflammation, and naturally this permeates his discussions of numerous special topics. Ricker and Oertel may be right, but many pathologists here and abroad do not think so. A book review is no place to argue the question, but the subject assumes indubitable importance in the mind of any teacher of pathology who advises his students in the selection of a textbook. Much the same criticism may be directed toward highly individualistic descriptions and classifications, such as those of Bright's disease.

With due consideration, and probably with the idea that specimens should be readily available to the student, Oertel has provided no pictorial illustrations. To be sure, a high grade laboratory should have plenty of illustrative material, but in not a few institutions the small volume of pathologic material and the cost of preservation of the material in suitable form are handicaps too great for the collection of those typical lesions which are so essential to the student's fund of information. Experience has shown that in nearly all fields of study the word picture, admirable as it may be, often requires amplification by illustrations. The value of a textbook on almost any subject is enhanced by well selected photographs, drawings and the like.

Although Oertel makes no claim for completeness, the references listed at the end of each chapter are well selected and on the whole reasonably extensive. Oertel is devoted to Germanic science and philosophy, a quality which is reflected in the numerous excellent summaries of what these peoples have contributed to pathology and medicine. Indeed, there is a suggestion of overemphasis on the work of Continental investigators in relation to the newer American school. The reviewer protests that in making this criticism he is not guided by undue nationalism but by a desire to see that all good things are included.

The format of the book is somewhat larger than is usual in this type of publication; the paper is of good mat surface, the type is large and the printing clear and uniform. The book is interesting and enlightening reading for those advanced students of pathology and medicine who are sufficiently experienced to formulate their own judgments of the expositions of a highly respected author.

Pathological Technique. A Practical Manual for Workers in Pathological Histology Including Directions for the Performance of Autopsies and for Microphotography. Frank Burr Mallory, A.M., M.D., S.D., Consulting Pathologist to the Boston City Hospital, Boston, Mass. Cloth. Pp. 434, with 14 illustrations. Price $4.50. Philadelphia: W. B. Saunders Company, 1938.

Receiving this book was just like meeting again an old friend whom one missed badly and for whose return one waited impatiently. The pleasure of the meeting was enhanced by evidences of rejuvenation and up-to-dateness, much more fundamental than a mere face lifting.

The reviewer's affection in approaching this book will be understood and, he hopes, excused by all who received their training in pathology during the past generation, when the names Mallory and Wright were synonymous not with a book on pathologic technic but with the subject of pathologic technic. When the book, only twenty-seven years old, reached in 1924, with its eighth edition, the enviable position of a standard in its field, the authors or the publishers did not respond to a general demand for a new edition, and soon the book could not be obtained. Other books were published, but none of them was able to replace the old standby. But here it is, dedicated to the memory of the former co-author, James Homer Wright.

It is a new book, though retaining all that was good in the old one. It has been brought up to date and considers all that has taken place since 1924, all that has proved valuable or that shows the promise to stay.

The book has gained by the changes to which it has been subjected; it has become truly a book on pathologic technic through the elimination of large chapters devoted to bacteriology, serology and hematology.

The subject matter is treated in three parts and nineteen chapters. The first part, 105 pages, deals with general histologic methods, including the examination of unfixed material and the processes of fixation, decalcification and embedding of tissues. The relatively new dioxane method is presented; then follow discussions of the stains, natural, artificial and metallic, of the clearing and mounting reagents, of microincineration and of injection methods. The special histologic methods as they are used for the study of the cell and of its component parts, of the different special cells and tissues and of the various organs are taken up in three chapters of the second part. The nervous system is treated exhaustively in 45 pages. Two chapters are given to bacterial stains and to infectious agents, such as actinomycetes, yeasts and molds, rickettsias, filtrable viruses, spirochetes, protozoa and worms. The sixteenth chapter of part three takes up the technic of the necropsy in 73 pages. The different methods are presented and their relative advantages discussed. This chapter contains a wealth of material in a limited space. Then follow brief and valuable chapters on the preservation and mounting of gross specimens, on gross and photomicrography and on the making of lantern slides, and finally with suggestions for such practical matters as the blackening of table tops and the cleaning of glassware. A well selected 8 page bibliography and a 28 page index conclude the book. The type and binding are satisfactory.

There is every reason to expect that this book will prove a worthy successor to the old "Mallory and Wright." No pathologist, no laboratory technician can afford to be without it. It is hoped that the author will see the editions multiply as he did in the case of the older book.

Books Received

NATIONAL RESEARCH FELLOWSHIPS 1919-1938. Physical Sciences, Geology and Geography, Medical Sciences, Biological Sciences. Pp. 95. Washington, D. C.: National Research Council, 1938.

LE DÉSÉQUILIBRE ALIMENTAIRE (CARENCE C.) DANS LES TROUBLES DU MÉTABOLISME CALCAIRE (OSSIFICATION ET DENTITION) EN PATHOLOGIE HUMAINE ET COMPARÉE. Géorges Beltrami, ancien interne des hôpitaux, professeur à la Faculté de Médecine de Marseille. Pp. 35. Marseille: M. Leconte, 1938.

HUMAN PATHOLOGY. A TEXTBOOK. Howard T. Karsner, M.D., Professor of Pathology, Western Reserve University, Cleveland. With an introduction by Simon Flexner, M.D. Fifth edition, revised. Cloth. Pp. 1013, with 461 illustrations. Price, $10. Philadelphia and London: J. B. Lippincott Company, 1938.

MANUAL OF VETERINARY BACTERIOLOGY. Raymond A. Kelser, D.V.M., A.M., Ph.D. Third edition, thoroughly revised. Cloth. Pp. 640, with 93 illustrations. Price, $6. Baltimore: Williams & Wilkins Company, 1938.

CANCER WITH SPECIAL REFERENCE TO CANCER OF THE BREAST. R. J. Behan, M.D., Dr.Med. (Berlin), F.A.C.S., Co-Founder and Formerly Director of the Cancer Department of the Pittsburgh Skin and Cancer Foundation, Pittsburgh. Cloth. Pp. 844, with 168 illustrations. Price, $10. St. Louis: C. V. Mosby Company, 1938.

CANCER. ITS DIAGNOSIS AND TREATMENT. Max Cutler, M.D., Associate in Surgery, Northwestern University Medical School; Chairman, Scientific Committee, Chicago Tumor Institute; Consultant, Tumor Clinic, and Director, Cancer Research, U. S. Veterans Administration, Hines, Ill., and Franz Buschke, M. D., Assistant Roentgenologist, Chicago Tumor Institute; Late Assistant, Roentgen Institute, University of Zurich. Assisted by Simeon T. Cantril, M.D., Director, Tumor Institute, Swedish Hospital, Seattle; Late Assistant, Chicago Tumor Institute. Cloth. Pp. 757, with 346 illustrations. Price, $10. Philadelphia and London: W. B. Saunders Company, 1938.